RELATIONS OF GERMINATING SOYBEANS TO TEMPERATURE AND LENGTH OF INCUBATION TIME

BY

THOMAS I. EDWARDS

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By THOMAS I. EDWARDS

Department of Biology, School of Hygiene and Public Health, The Johns Hopkins University
[*Botanical Contribution No. 118 from the Laboratory of Plant Physiology, The Johns Hopkins University*]

INTRODUCTION: TWO CYCLES OF PUBLICATIONS

THE literature on the temperature relations of seed germination extends throughout about a century and a third and may be considered as of two cycles: from 1860 to 1878 and from 1904 to the present. Precise study of these relations could not begin, of course, until after the invention of the thermometer, and a good many years elapsed after its invention before that instrument found use in seed-germination studies. During the nineteenth century there were two main reasons for studying this subject; one was the purely utilitarian problem of learning how to make crops grow better, but every worker considered his results in relation to the problem of plant distribution. Certain kinds of plants were known to be naturally restricted to tropical regions, for instance; was this due to the necessity for high temperatures during germination? Conversely, were plants of the temperate zones prevented from growing in the tropics because of high temperatures? Seed-germination studies seemed to promise valuable evidence on these and other ecological questions, which undoubtedly provided a powerful stimulus for studies of this kind. Automatically controlled temperatures were not available and workers utilized selected locations in cellars and greenhouses, where temperature fluctuation was slight, to provide the lower temperatures they needed. For higher temperatures pots of soil or metal chambers were heated by means of small oil flames, the distance of the flame below the vessel being chosen so as to give the required temperature.

Lefébure (38) published on this subject in 1801 and Edwards and Colin's paper (15) appeared in 1834. Sachs's (48) clear discussion of the general temperature relations of seed germination, which was published in 1860, seems to have aroused considerable interest in this subject. Alphonse de Candolle (10), who may have been somewhat influenced by Sachs, published in 1865. In 1874 and 1875 Friedrich Haberlandt (25, 26) contributed two important papers, which were so thoroughly and carefully executed that they appear to have removed the incentive for further work in this field until about the end of the nineteenth century. At about the same time as Haberlandt's work (namely in 1870 and 1878) there appeared two papers by de Vries (11) and by Oudemans and de Vries (46). That some kinds of seeds may germinate at the temperature of melting ice was shown at about this time by Uloth (52), whose papers were published in 1871 and 1875, as well as by de Candolle (10) and Haberlandt (27), in 1865 and 1875, respectively. The short period characterized by the work of Sachs, de Candolle, de Vries, Haberlandt and Oudemans and de Vries may be considered as ending in 1878 and there followed twenty years without important publications on this topic.

A new cycle of activity in the study of seed germination as related to temperature opened about the beginning of the present century and has continued to the present, with an accelerated increase in the frequency of contributions. By this time a new situation had arisen, with new motives for studies of this kind. For one thing, there was a growing interest in physiological temperature relations in general, and tests on seeds afforded a means for studying optimal temperatures and for inquiring into their significance. Interest in the 10-degree temperature coefficient (Q_{10}) prompted several studies on germinating seeds. For practical purposes it became desirable to know the most satisfactory temperatures for carrying out seed viability tests. Interest in such problems as those of after-ripening and light sensitivity led several workers to study temperature influence on germination. On the whole, the ecological aspects of germination studies seem to have been lost sight of, and it appears that the work of this cycle is largely independent of that of the first, for only a few of the twentieth-century authors give much attention to accounts published in the first cycle.

Some features of the trends just discussed are brought out by the following tabulation of the numbers of papers on this subject that appeared in successive five-year periods.

PERIOD	NUMBER OF PAPERS	PERIOD	NUMBER OF PAPERS	PERIOD	NUMBER OF PAPERS
1860–64	1	1885–89	—	1910–14	3
1865–69	1	1890–94	—	1915–19	2
1870–74	2	1895–99	—	1920–24	8
1875–79	2	1900–04	1	1925–29	19
1880–84	—	1905–09	2	1930–	5

In this table the two cycles of publication stand out clearly with a 26-year interval between them. There is a remarkable regularity of increase in the second cycle, especially when it is realized that the literature on this subject is so scattered that few workers are able to cite more than four or five papers dealing with the temperature relations of seed germination. The peak of publication appears to fall in the period 1925–29, more specifically in the two-year period 1928–1929, and articles are now appearing at a slower rate. A careful search of many bibliographic indexes and abstracting journals has failed to show any important papers on seed germination published in the interim between 1880 and 1900, and later writers on this subject do not cite any important contributions that appeared in that period. Fashion turned away from this kind of seed germination study about 1880 and did not return till after 1900. Such changes of fashion in botanical research have recently been discussed in an interesting way by Stevens (50).

The following sections present a review of the literature on the temperature relations of seed germination as shown by experiments in which maintained temperatures were employed. The subject of temperature fluctuation or alternation, specific effects of which have been reported for a number of kinds of seed, is not included in this review. Another special subject not taken up here deals with the after-ripening of seeds and the causes of delayed germination. There is a considerable literature on each of these aspects of seed germination.

This review was prepared in connection with an experimental study of the influence of maintained temperatures on the germination of a lot of soybean seed (14), carried out at the Laboratory of Plant Physiology of the Johns Hopkins University, and the writer has had the benefit of critical suggestions and editorial help

from Professor Burton E. Livingston, of that laboratory.

LITERATURE OF THE EARLIER CYCLE

Some of the earlier papers on temperature and seed germination that seem to be most important are briefly reviewed in chronological order in the following paragraphs. In 1801 E. A. Lefébure, of Strasbourg, published (38) a book on the germination of radish seed. His work was remarkably thorough and included most of the lines of study that are being followed today in the investigation of seed germination, including the incubation of seeds at a number of approximately maintained temperatures. Lefébure's seeds were killed by exposure to 40°–45° C. and at 36°–38° only one out of 30 seeds germinated but the others were uninjured. At 32°–34° the greater part of the sample began to germinate within 72 hours; at 28°–30° germination was still more rapid and at 22°–25° about 48 hours of incubation was usually required. For temperatures between 12° and 25° little difference in time of germination was noted, these tests being made out-of-doors at suitable times. At 7°–8° germination was slow and at 5°–6° no seedlings were found after 5 days. When seeds were incubated under water the temperature relations were essentially like those just outlined, but longer incubation periods were necessary when water was used.

Lefébure supposed that "caloric" was a specific stimulant for organized bodies; below a certain intensity (8°) it was impotent to stimulate the embryo; from 8° to 38° it was effective, but still greater intensities were injurious. An early concept of physics, which has since been abandoned, and a terminology developed subsequently are the only features that render Lefébure's way of thinking seriously different from ours at the present time.

As early as 1834 W. F. Edwards and M. Colin (15) published a paper in which they set forth some important generalizations that still hold. Their numerical data are fragmentary and their technique would hardly be employed now without considerable modification, but they showed a remarkably clear appreciation of the temperature relations of germinating seeds. They said, for example, that

> . . . . la germination doit s'accélérer avec l'accroissement de la chaleur; mais d'abord les différences dans les vitesses de germination de 10° en 10° de température ne sont pas aussi grandes que l'on pourrait le croire. Et en second lieu, il se passe un phénomène remarquable lorsque la température se rapproche de la limite supérieure. Les graines qui jusqu'alors avaient germé plus vite à mesure que la température était plus élevée, non-seulement s'arrêtent dans cette progression, mais encore rétrogradent; c'est-à-dire qu'aux plus hautes températures où elles peuvent germer, leur germination éprouve un retard considérable.

In 1860 Sachs (48) published an account of a study in which he germinated seeds of corn, wheat, barley, cucumber and scarlet-runner bean in soil at several different temperatures—which were not maintained very exactly—and gauged the efficiency of the temperature by the length of root or shoot produced in 48 hours. His discussion is specially valuable. He defined minimal, optimal, and maximal temperatures for the first time and called attention to the fact that the minimal temperature is generally farther removed from the optimum than is the maximum.

Alphonse de Candolle (10) gave special attention to the time relations of seed germination at different and more or less well maintained temperatures between about 0° and about 50°–57°. He experimented with seeds of 12 species, germinating small samples (usually from 10 to 30 seeds) in moist sand. For each species the time required for the appearance of the first seedling was shorter as successively higher temperatures were examined. At

28°, however, several kinds of seeds required a longer time to germinate than at 24°. De Candolle chose the time required for the appearance of the first seedling in a sample as a criterion for evaluating the influence of temperature on germination, but he recognized that there was much variation in this time requirement.

In 1870 de Vries (11) repeated Sachs's experiments, using a wider range of maintained temperatures and more kinds of seeds. Sachs's experiments were also repeated by Friedrich Haberlandt (25, 26) whose very important reports appeared in 1874 and 1875. In tests that included the seeds of about 70 agricultural plants he found that the seeds of most of the forms tested gave high germination percentages at 19°, 25° and 31°, but that some kinds of seeds showed low germination percentages at higher temperatures. Several kinds gave some germination at 44° but no germination was observed at 50°. In order to specify which of the temperatures tested were most favorable for germination Haberlandt found it necessary to examine the time relations of germination. Taking as a basis the incubation time elapsing before the appearance of the first seedling in any culture, he found that high and low temperatures retarded germination and an intermediate range of temperature permitted germination in a minimum of time. He regarded as optimal the maintained temperature at which germination began to occur in the shortest time. The minimal and optimal temperatures for seeds of tropical crop plants were found to be higher than for the crop plants of temperate regions but their maximal temperatures were no higher.

Oudemans and de Vries (46) germinated seeds of 16 species of cultivated plants in soil, in greenhouse rooms with temperature ranges from 7°–8° to 15°–21°. Minimal germination temperatures were shown for several species. The lengths of time required for the appearance of the first and last seedlings were found to be shorter as the temperatures were higher but these tests did not include any temperature range so high that germination appeared to be retarded.

LITERATURE OF THE LATER CYCLE

Reynolds (47) germinated the seeds of a number of crop plants at five temperatures between 10° and 32°, noting the germination percentages for several lengths of incubation period. Wheat, maize, and turnip seed received special attention.

For temperature control Atterberg (3) used apparatus somewhat similar to the battery of chambers constructed and described by Livingston and Fawcett (40) but without stirring apparatus. He studied the influence of temperature and incubation time on germinating seeds of wheat, barley, rye, and oats. The seeds were examined at two-day intervals as they lay in folds of linen cloth, and the number of germinated seeds was recorded at each observation. Atterberg's data for oats (3, p. 140, and 39, p. 406) may be used to illustrate several relations that are frequently found in temperature-germination studies. In figure 1 germination percentages are ordinates, constant temperatures are abscissae, and each length of incubation period is represented by a separate graph. For the shortest incubation period, germination was confined to the narrowest temperature range and with successively longer periods the range within which germination occurred became progressively broader. For four days of incubation 19° gave the highest germination percentage and it might properly be called the optimal temperature for that period; for six days the optimal temperature shifted to 15°. Although

this sort of regression of the temperature optimum has been reported by Borthwick (6), Haasis (23, 24), Fawcett (17), Tang (51), and others, many sets of published data show no signs of such a shift. Another notable feature of figure 1, which is common to most such sets of graphs, is a pronounced broadening of the optimal-temperature range to include progressively lower temperatures with longer incubation.

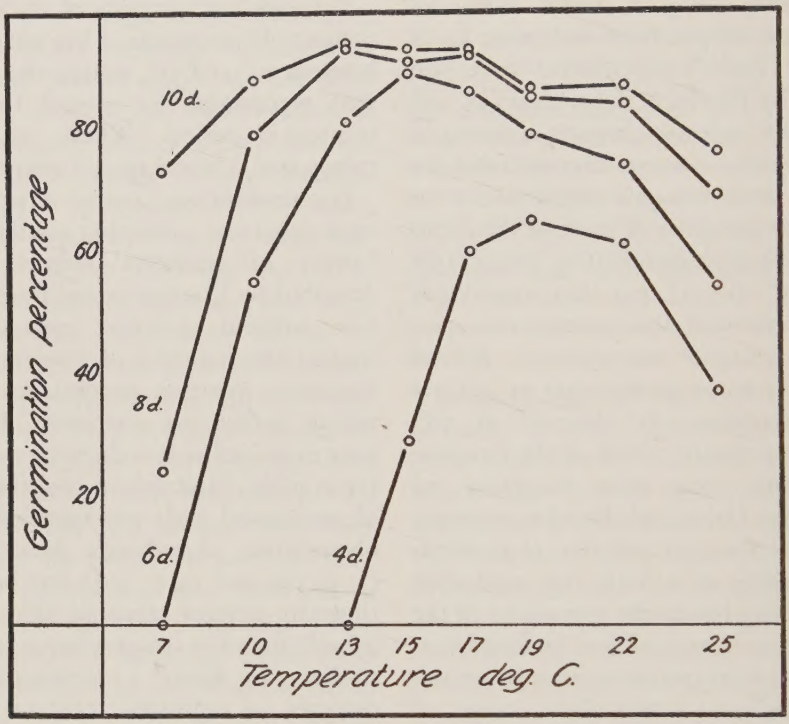

FIG. 1. THE RELATION OF THE GERMINATION OF OATS TO TEMPERATURE
For explanation see text

For all the kinds of seeds tested by Atterberg the germination percentage eventually approached 100 for the lower temperatures, but for temperatures above a critical magnitude the final percentage was progressively smaller with progressively higher temperature. The critical temperature just mentioned was considered by Atterberg to be optimal; it represents the highest temperature at which seedling production was unimpaired. When interest is focussed on securing the maximal number of seedlings from a lot of seed, without reference to the length of incubation, this sort of optimal temperature is more useful than the one defined as the temperature inducing the most rapid production of seedlings.

Atterberg's data for wheat seed six months old show an optimum near 15°; several later workers have reported a similar optimal temperature but others, including some of the earlier workers, find optima nearer 30° than 20°. The extreme variation (15°–30°) in values for the temperature optimum for wheat suggests that specially planned studies will be necessary before the discrepancies already reported may be understood. The work of Wilson and Hottes (54) indicates that

wheat of the same variety grown under diverse climatic conditions has the same temperature relations and that different varieties have about the same responses. The age of the sample, however, is very important; the work of Harrington (31) and Munerati (43, 44) has shown that freshly harvested wheat seed has a low temperature optimum and that during storage it acquires the ability to germinate well at higher temperatures. Atterberg (3) observed similar behavior in barley. This deserves further study, as does the influence of different substrata and various experimental procedures. Such studies might well include not only a broad range of maintained temperatures with short temperature intervals but also short intervals of observation.

Gassner (18, 19) examined the temperature relations of germination for a number of seeds, most of them being light-sensitive or requiring alternating temperature for best germination.

The results of Akemine (1) on the temperature relations of germinating rice seed are representative of those of several workers who studied rice seed—Haberlandt (26), Ocfemia (45), Winkler (55), Jones (33),—and they agree in general with the results reported by Livingston and Haasis (41). The minimum is shown as about 10°–12° and the maximum as about 40°–45°, according to the variety of rice studied and the conditions of incubation. Temperatures between about 15° and about 36° permitted almost complete germination of the samples if sufficient time were allowed, although the rapidity of germination depended upon temperature. Perhaps there is no kind of seed for which the reports of different workers are so nearly in accord.

In the course of experiments on temperature relations of nuclear phenomena Hartmann (32) made some preliminary germination tests at eight constant temperatures between 8.5° and 41°, using moist sawdust as substratum. For seeds of *Phaseolus multiflorus*, *Helianthus annuus*, maize, and pea, 31° induced the earliest appearance of seedlings; at higher and lower temperatures seedlings appeared later.

Harrington (29) carried out temperature studies on seeds of a number of plants cultivated as ornamentals, to gain information concerning the best temperatures for use in seed testing for these forms. For the species tested (of which there were about 18) 15° and 22.5° yielded higher germination percentages than were given by higher temperatures. In a later paper (30) the same writer noted that for several other kinds of seed (Bermuda grass, celery, and Johnson grass, for example) temperature alternation gave far greater germination percentages than did any of the maintained temperatures tested.

Clytee Evans (16) germinated seed of *Amaranthus retroflexus* at nine temperatures between 9° and 46°. The range 21°–42° may be regarded as optimal for rapid production of seedlings, 42° giving most rapid germination.

Coffman (9) incubated seed of about 30 crop plants at several temperatures between 0° and 14°–17°. The seeds were between sheets of wet blotting paper and the temperatures were only approximately maintained. His data show very marked differences among the varieties tested, with respect to minimal temperature for germination; seed of the cereals, clover, alfalfa and several cruciferous plants germinated well at the lower temperatures used, even at 0°.

Dickson (12, 13) germinated seed of several varieties of spring and winter wheat in soils that were maintained at eight different temperatures between 8° and 36°, but he presented no tables or

graphs. He wrote (12, p. 69): "The rate of emergence increased gradually with the rise of soil temperature until at 28° the seedlings reached the surface in from 38 to 48 hours. Above this soil temperature the rate of emergence decreased very rapidly." From this statment, 28° might be regarded as the optimal temperature for the germination of his lot of wheat seed, but in the same paper it is stated that seed of Turkey wheat gave average germination percentages above 95 up to and including a soil temperature of 20°; above 20° the germination percentages were smaller as higher temperatures were used. The performance of Marquis wheat was similar to that of the Turkey variety. According to the criteria used for judging it, the optimal temperature might be set either about 20° or about 28°. Referring to seedling growth, Dickson noted that at 8° and 12° roots developed to a considerable extent before shoots showed much growth, while at a temperature of 28° or higher the tip of the shoot was well above the soil surface before root development became pronounced.

Kreysing (37) and Bär (4) incubated seed of several Festuca species at different constant temperatures between 4° and 33°, noting the germination percentages for various lengths of incubation period and the average incubation periods required for germination at the several temperatures. At 30° and 33° most samples required longer incubation than at the next lower temperature tested.

Borthwick (6) germinated asparagus seed on moist flannel at 10°, 20°, 25°, 30°, 35° and 40°. In the 17 days of his test period no seeds germinated at the highest or at the lowest of these temperatures. For the first four or five days 30° was most favorable for seedling production; after eight days 25° yielded higher germination percentages than either 20° or 30°, but with still longer incubation 25° and 30° gave the same percentage values. From Borthwick's data graphs may be constructed which show a high degree of consistency and are very instructive.

In the course of extensive experiments on conditions affecting seed germination, Morinaga (42) germinated seed of white clover, celery, and water cress under water and on moist filter paper, at seven different maintained temperatures that ranged from 5° to 38°. Seed of white clover and water cress germinated better under water than on moist paper, at all tested temperatures but celery seed germinated at a higher temperature on moist paper than under water. The optimal temperature range for white clover seed broadened from 22°–27° (for a two-day period) to 10°–32° (for a 10-day period). The range 10°–15° yielded the best germination percentages for celery and the range 15°–22° was most favorable for seed of water cress.

Munerati (43, 44) found that newly harvested wheat seed gave low germination percentages at temperatures of about 22°, but percentages as high as 99 were obtained with the temperature range of 5°–7°. This agrees with the findings of Harrington (31). In Munerati's tests a sample of wheat seed that had been stored for a year germinated as completely at 22°–24° as at 12°–14° or at 5°–7°, but only 47 per cent of the seeds germinated at 30°–32°. Wheat stored two years gave low germination percentages, the lowest being for the lowest temperature range tested. Similar results were obtained with other varieties of wheat and other cereals, agreeing with Atterberg's (3) similar findings for barley seed. Differences in germination of samples from different years were so marked that Munerati proposed germination tests at various temperatures as a means of ascertaining the ages of different lots of seed.

Kotowski (36) recorded for each kind of seed tested not only the final germination percentage but also the number of days elapsing before the appearance of the first and the last seedling in each culture, and introduced a coefficient of germination velocity to represent the rate of seedling production. His data and his treatment of them are specially valuable. Seeds of 17 kinds of garden vegetables were germinated in soil at six maintained temperatures between 4° and 30°. For all the varieties studied the incubation time required for the appearance of seedlings was greatest for the lowest temperature tested and became progressively shorter for progressively higher temperatures. This is shown not only by his data for the times of appearance of the first and last seedlings but also by his coefficients of velocity. For spinach, the highest germination percentage is recorded for 4°, which agrees with Sifton's (49) findings. The following quotation is from page 181 of Kotowski's publication: "The optimum for cabbage was 8°C., and for beet 11°C. The advantage of low temperatures may not be significant for cauliflower, carrot, and parsley, the number of seedlings being equal within intervals 8°–11°–18°C. For pea and onion 18°C. was the best, for lettuce, 25°C. and for tomato the range 18°–30°C. was very favorable. The other species employed did not give exact indications upon their optimal points for germination, they probably lay at 30°C. or higher."

Borthwick and Robbins (7) secured final germination percentages of 98 or 99 for lettuce seed incubated on moist flannel at six temperatures between 1° and 25°. Their main lot of seed had been stored 18 months after harvest. For 27° and still higher temperatures the percentage value was much lower than for 25°, which may therefore be regarded as optimal. Kotowski's (36) results with lettuce seed also show this relation. For Borthwick and Robbins's main lot of lettuce seed the highest temperature permitting germination was found to be 29°, but for lots of seed more recently harvested the optimal and maximal temperatures were lower. These writers tested seed of 20 varieties of lettuce and some of the samples used germinated at 29°, but the main lot of seed had integuments of such nature that they apparently limited gas exchange and thereby prevented germination at 30° unless special precautions were taken—such as incubation in an atmosphere rich in oxygen or preliminary soaking at 4° for 4–6 days.

Grimm (22) tested the germination of clover seed in moist sand at four different temperatures. After three or ten days of incubation, cultures at 30° were found to have the highest germination percentages; 20° was slightly inferior in this respect, and 10° and 40° yielded still fewer seedlings, being about equally effective.

Wilson (53) studied the temperature relations of seed germination for three varieties each of wheat and soybean and for one variety of oats, incubating his seeds on moist plaster-of-Paris blocks. For soybean as well as for oats the final germination percentages were not very different for temperatures of 10°, 15°, 20°, 25°, and 30°. His wheat seed did not give as large germination percentages at temperatures above 20° as at lower temperatures, which is essentially in agreement with Atterberg's (3) and Dickson's (12) findings for wheat seed. Different lots of seed of the same variety, but produced in different regions of the United States, exhibited no considerable differences with respect to the temperature relations of germination.

Haasis (23) conducted a series of experiments on the germination of lots of coniferous-tree seeds as related to temperature

and time. Seed of pitch pine (*Pinus rigida*) and some other species began to germinate only at two separate temperature ranges and two optimal temperature ranges consequently appeared for the shorter incubation periods. The proportion of seeds able to germinate at the higher temperatures was small, however, and lower temperatures were much more favorable for the germination of most of the seeds of the lot; but the process went on at a slower rate as the temperature was lower. As a result, with increasingly long incubation periods the optimal temperature range shifted to lower temperatures and the double optimum shown for the shorter incubation periods disappeared. Haasis's seeds of pitch pine tolerated a very wide range of maintained temperatures; at the end of 14 days of incubation some seedlings had appeared at 16° (the lowest temperature tested) and 12 per cent had germinated at 57° (the highest temperature tested); the largest germination percentage (91) was given by 24° maintained 14 days. Unlike most other sets of graphs representing physiological temperature relations, those of Haasis are steepest on the low-temperature side. The temperature giving the greatest germination percentage for a given length of incubation period is taken as the optimal temperature for that length of period. The maximal temperature and the minimal temperature for any length of period are taken as the highest and lowest temperature at which any germination was observed in the specified period. All three of these critical temperature values differ greatly with differences in length of incubation time. For seeds that germinate promptly, such as those of most agricultural plants, the influence of incubation time in modifying the positions of the three cardinal temperatures is easily overlooked unless observations are sufficiently frequent. When germination is slow, however, as in pine seed, the importance of the time factor is readily apparent without specially frequent observations. Haasis called attention to the possibility of sorting or selecting seedlings for subsequent experimentation, according to the time-temperature complex that prevailed during the germination period. This possibility has received further attention from Edwards (14) and from Livingston and Haasis (41).

Meta Bihlmeier (5) studied the relations to temperature and light of germinating seeds of 10 species of the Labiatae and Cruciferae, finding that the optimal temperatures derived from tests with and without light were the same for all forms tested. Optimal temperatures were judged by two criteria; they gave both large germination percentages and high rates of germination.

Fawcett (17) germinated seeds of sweet orange and sour orange in jars of sand kept at nearly constant moisture content and incubated at nine maintained temperatures between 12.6° and 40.0°. His observations extended throughout a period of 105 days. Sweet-orange seed germinated most rapidly at 33° in the first 40 days, but temperatures from 23° to 33° yielded about the same germination percentages with longer incubation. For the 105-day period the minimal and maximal temperatures appear to have been close to 12.8° and 40.0°, respectively. Sour-orange seeds germinated at all temperatures tested except 40°, but in the first 40 days the largest germination percentages were found for 29°. For progressively longer periods the optimal temperature range was extended to lower temperatures more rapidly than to higher ones. After germination the resulting seedlings were grown in light at the same temperatures, and measurements of

seedling height were made. For 30 days 33° appears to have yielded the tallest sour-orange seedlings; for 40 days 26° and 29° were optimal in this respect and after 90 days 23° and 26° were the temperatures giving the best-looking plants. For sweet orange, too, the optimal temperature for seedling growth was lower than the temperature inducing most rapid germination.

Hilda Joseph (34) found exceptionally high minimal temperatures for the germination of birch seed, even after a month's dry storage. Seed of *Betula lenta* germinated only at 32°, the highest temperature tested; that of *B. papyrifera*, *B. populifolia*, and *B. lutea* germinated at 20°, 25°, and 32°, but not at 15°. Alternation of temperature did not increase the germination percentages. Storage in the moist condition at 0° and 5° for two weeks or more so altered the seeds that they afterwards germinated about equally well over a broad range of temperature. The temperature relations of germinating parsnip seed were found by the same author (35) to be altered after prolonged storage.

Bremer (8) germinated beet seed at a large number of temperatures between 3° and 42°, recording the time required for the appearance of the first radicle. The range 22°–36° appears to be optimal, the maximum and minimum fall outside the temperature range tested.

Gericke (21) incubated wheat seeds at the surface of 126 different three-salt solutions at each of seven maintained temperatures between 13° and 35°. All the solutions tested gave about the same germination and early growth and all solutions were superior to distilled water. Temperatures of 28° and 31° yielded the longest seedlings after 110 hours of incubation.

Haasis and Thrupp (24) germinated lodgepole pine (*Pinus contorta murrayana*) seeds on agar at seven maintained temperatures, ranging from 15° to 41°. Their seed samples were collected in British Columbia, from six localities that differed in altitude so greatly as to represent a wide range of ecological conditions. At 19° seeds from the highest elevation showed much better germination than those from low elevations but at 41° seeds from low elevations yielded higher germination percentages than seeds from higher localities. For intermediate temperatures the place of collection exerted no apparent influence on the percentages. As in Haasis's earlier contribution (23), a double temperature optimum was observed for all six samples.

Tang's (51) wheat seeds were submerged in dilute mineral nutrient solution continuously aerated and stirred by means of a bubbling stream of air. He employed seven maintained temperatures and several different degrees of aeration. The optimal temperatures for germination with different combinations of temperature, aeration and time were found to be very different. Depending upon the environmental treatment and the length of the incubation period, cultures at 35°, 30° and 24° were all optimal, the highest temperatures being in combination with low degree of aeration and a short incubation period. Tang emphasizes Haasis's (23) suggestion that germination tests may be valuable in the selection of seedlings for experimentation on later phases of growth.

Lehmann and Aichele's recent review (39) of literature and summary of present knowledge concerning seed germination in the Gramineae constitutes a very valuable work, which will be used by students of seed germination for a long time.

In the course of experiments on the growth of oats Hamada (28, p. 169) made germination tests at four temperatures.

Twenty-four hours sufficed for the appearance of the first seedlings at 24° or 28° but more than twice as much time was required at 36° or 16°. Haberlandt (25) also found earlier germination of oats at 25° than at 16° or at 31°, but Atterberg's (3) results (already mentioned) show different relations.

Gäumann (20) presented the results of Tanja, in part, on the length of time required for wheat seedlings to attain a length of 5 cm. at temperatures between 3° and 36°. At 27° the least time (about five days) was required, confirming Dickson's findings (12, 13); a longer incubation period was required at both higher and lower temperatures—about seven days at 36° and forty-two days at 3°.

Edwards (14) incubated soybean seed on agar plates at five maintained temperatures between 24.5° and 40.0°, making counts of germinated seeds at two-hour intervals. At 33.0° and at 36.5° (a) the least time was required for the appearance of any given germination percentage, (b) the greatest percentage of seeds germinated in any of the incubation periods tested, and (c) the mean incubation time was shortest. The rate of seedling production showed a maximum for the third two-hour interval in which any seedlings appeared. For every one of the five temperatures tested the germination percentage attained when the rate of seedling production began to fall off was found to be 35–40 per cent. With additional time the rate decreased more slowly than it had increased prior to the attainment of its maximal value. Removal of germinated seeds from test cultures at regular intervals was emphasized as constituting a sorting process, distributing the seedlings of a lot into several classes according to their rates of emergence under specified uniform and favorable conditions; the increments of germination percentage from one observation to the next thus indicate the relative sizes of these physiological classes in the original seed sample as well as in the resulting lot of seedlings. This sort of physiological test might be employed when a number of similar seedlings are wanted for experimentation on later phases of development, as was suggested by Haasis (23) and Tang (51) and discussed to some extent by Livingston and Haasis (41).

THE CONCEPT, OPTIMAL TEMPERATURE FOR SEED GERMINATION

The literature on temperature relations of seed germination includes accounts of a number of experimental studies carried out with the purpose of selecting the best maintained temperature for use in viability tests, which constitute a large part of the work of seed-testing laboratories (2). Some accounts deal more directly with the fundamental question of temperature optima for germination, a question that is naturally closely related to minimal and maximal temperatures. With somewhat different aims and with a great variety of experimental procedures, different writers in this field have employed the expression *optimal temperature* with markedly different meanings, some of which may be mentioned in illustration.

(A) Many workers have considered as the optimal temperature for germination the upper limit of a range of temperatures at which approximately the same final percentage of germination is attained when tests are continued long enough to make sure that the maximal germination percentage has been attained.

(B) For a given length of incubation period an optimal temperature may be taken as the temperature yielding the greatest germination percentage at the end of the period. The physiological significance of an optimum of this sort naturally

depends on the length of the incubation period chosen as base for comparisons among the tested temperatures, and the same lot of seed may thus show a number of widely different optima.

(C) Some writers have been specially interested in the time required, at each tested temperature, for the first appearance of any seedlings in a sample of seed and for the appearance of the last seedling. On this basis the optimal temperature may be taken as the temperature at which the least time is required for the appearance of the first or of the last seedling; generally the resulting optimal temperature is the same whether the first or the last seedling is employed. This kind of optimum was the most popular with the earlier workers in this field, frequently it was the only measurement recorded. It has been little used in the last decade, except as supplemental to records of germination percentages. Since the extreme limits of variation with respect to promptness of germination, on which this concept of temperature optima is based, are apt to vary with the number of seeds in the test-sample, it is clear that the size of the sample is important; it should always be relatively large. If a larger and a smaller sample from the same lot of seed are incubated under the same environmental conditions, some of the seeds in the larger sample may be expected to germinate more promptly than any of those in the smaller sample, but if both samples are sufficiently large this kind of difference should not appear. For this reason, as well as for other reasons, results of germination tests that are published without reference to the size of the seed samples employed may be very unsatisfactory.

(D) In some instances the optimal temperature for germination has been taken as the temperature giving the largest average height (or the greatest degree of vigor, etc., estimated in some suitable way) of the seedlings of a set at the end of a specified period for germination and subsequent growth. Such an optimum must refer specifically to the given standard time period, for tests with different lengths of period may of course lead to the choosing of different temperatures as optima. Furthermore, the employment of any criterion of seedling vigor necessitates a confusing combination of the germination process itself with the subsequent enlargement and development of the resulting seedlings; seed germination and seedling growth can hardly be presumed to be related to temperature in exactly the same manner, however germination may be defined.

(E) The temperature corresponding to the least average incubation time may be taken as optimal for the sample, since the behavior of all individuals in the sample is thereby taken into account.

(F) Some authors consider both the mean rate of seedling production and the final germination percentage, when the general temperature optimum for seed germination is to be estimated.

(G) A smoothed temperature curve of germination percentage is sometimes fitted to the plotted points and the temperature corresponding to the peak of this curve is taken as optimal. Considering the great amount of variation in the temperature relations reported by different workers using seed of the same species or variety, and bearing in mind the great importance of experimental technique in the derivation of critical temperature values, etc., it appears that there is as yet no need for such refinement of method and statement.

Whatever may be the criteria and technique employed for fixing upon optimal temperatures for seed germination, the result necessarily involves some standard by which the concept of germination itself

may be limited. All would probably agree that germination represents the earliest stages of renewed growth and development that finally transform a seed into a seedling, but the end of the germination process may be variously defined according to the nature of the study in question or according to the viewpoint of the experimenter. Some writers regard a seed as germinated when the seed coat bursts; others consider a seed as germinated only after the root or shoot has emerged or after shoot or root, or both, have attained a considerable length.

CARDINAL TEMPERATURES FOR SEED GERMINATION

The temperature optimum

For the seeds of most crop plants the optimal temperatures for germination usually fall between 20° and 30° and directions for seed testing (2) usually specify one of these temperatures for incubation. Few optimal values above 30° have been reported; in fact, it is not often that higher temperatures have been tested. When both high final germination percentage and rapidity of germination are taken into account in defining the optimum, it appears that the optimal temperature for maize, rice, and soybean seed lies near 35°. On the other hand, several workers have reported that wheat germinates best at 15°. For several rosaceous seeds that undergo after-ripening 10° or lower may be optimal. Such seeds usually have other physiological peculiarities.

The temperature minimum

Like other physical and physiological processes, minimal temperatures for seed germination are somewhat difficult to establish experimentally. The lowest temperatures at which germination has been observed have shown very slow responses, requiring long incubation periods. It therefore follows that failure of germination to occur at a low temperature and within a given period does not necessarily imply that germination might not be possible with still more prolonged incubation. Although most students of seed germination have not studied minimal temperatures as thoroughly as optimal and maximal temperatures have been studied, nevertheless a number of different kinds of seed have been reported to show considerable or even high germination percentages after sufficiently prolonged incubation at about 0° and under otherwise suitable conditions.

Of course not all failures to secure germination at a low temperature may be ascribed to too short incubation. Although several workers have tested rice seeds at temperatures below 10° germination at such low temperatures has so far not been reported, apparently. A temperature of 14° appears to be too low for the germination of melon seeds.

Of special interest in this connection are the observations of de Candolle (10), Uloth (52), Haberlandt (27), and Coffman (9), on the germination of seeds when in contact with ice. De Candolle reported germination at 0° for white mustard and white clover. Uloth found well developed seedlings of wheat and of *Acer platanoides* in an ice cellar at Bad Nauheim in May, where the temperature presumably had not been above the melting point of ice since the preceding December, when the seeds had accidentally been placed in storage along with the blocks of ice. Wheat roots, for example, had penetrated through half a foot of ice and had grown several inches beyond. Each root was in a channel somewhat larger in diameter than the root itself, which suggested that the growing roots had actually melted their way through the ice, probably by means of heat liberated in respiration.

These observations led Uloth to carry out germination experiments with 25 different kinds of seed. His seeds were on or between blocks of ice with or without soil, and his tests lasted five months. Among common crop plants, seeds of wheat, barley, rye, oats, and pea gave medium or high germination percentages at the end of four months of incubation and seeds of radish, spring cress, white mustard, and hemp gave similar results.

Haberlandt obtained no germination of seeds of wheat, barley, oats, or white clover on ice, after an incubation period of four months; but he reported germination in seed samples of rye, pea, hemp and red clover, after 45 days on ice. He also obtained considerable germination of white mustard and alfalfa seeds on ice and an average root elongation of nearly 1 cm.

Coffman's experiments with seeds incubated on ice lasted only 12 days, but in that short period he obtained germination percentages between 9 and 32 for wheat, barley, rye, alfalfa, and radish. His seed of red clover gave a germination of only 1 per cent in 12 days on ice, whereas Haberlandt reported for this species a germination of 10 per cent after four months of incubation on ice.

The temperature maximum

There have not been many attempts to ascertain the upper temperature limits for germination. Haasis (23), however, reported that some seeds of *Pinus rigida* germinated when incubated at 57°C. De Candolle (10) observed that seeds of *Sesamum orientale* germinated when the incubation temperature fluctuated between 50° and 57° but Haberlandt (26), working with the same species, obtained no germination at 45° although he, too, reported germination at 40°. *Amaranthus retroflexus* has germinated at 46° (16). Rice appears to germinate at 42° but not at 45° (1, 41).

INDEX TO THE LITERATURE ACCORDING TO PLANT FORMS

The studies already noted may be brought together according to the kind of seeds used. In this list are included only kinds of seeds which more than three workers tested.

Wheat. Sachs, 1860 (48); Uloth, 1871, (52); Haberlandt, 1874 (25); Reynolds, 1904 (47); Atterberg, 1907 (3); Coffman, 1923 (9); Harrington, 1923 (31); Munerati, 1925–26 (43, 44); Wilson and Hottes, 1927 (54); Wilson, 1928 (53); Gericke, 1929 (21); Tang, 1931 (51); Gäumann, 1932 (20).

Oats. Haberlandt, 1874 (25); Uloth, 1875 (52); Oudemans and de Vries, 1878 (46); Atterberg, 1907 (3); Coffman, 1923 (9); Wilson, 1928 (53); Hamada, 1931 (28).

Maize. Sachs, 1860 (48); de Candolle, 1865 (10); Haberlandt, 1874 (25); Oudemans and de Vries, 1878 (46); Reynolds, 1904 (47); Hartmann, 1919 (32); Coffman, 1923 (9).

Rice. Haberlandt, 1875 (26); Akemine, 1914 (1); Harrington, 1923 (30); Ocfemia, 1924 (45); Jones, 1926 (33); Winkler, 1926 (55); Livingston and Haasis (41).

Barley. Sachs, 1860 (48); Haberlandt, 1874 (25); Uloth, 1875 (52); Oudemans and de Vries, 1878 (46); Atterberg, 1907 (3); Coffman, 1923 (9).

Peas. Sachs, 1860 (48); Haberlandt, 1874–75 (25, 27); Uloth, 1875 (52); Oudemans and de Vries, 1878 (46); Reynolds, 1904 (47); Hartmann, 1919 (32); Harrington, 1923 (30); Kotowski, 1927 (36)

Beans. de Vries, 1870 (11); Haberlandt, 1874 (25); Uloth, 1875 (52); Oudemans and de Vries, 1878 (46); Reynolds, 1904 (47); Hartmann, 1919 (32); Harrington, 1923 (30); Kotowski, 1927 (36).

Alfalfa and Clovers. de Candolle, 1865

(10); Haberlandt, 1874–75 (25, 27); Oudemans and de Vries, 1878 (46); Coffman, 1923 (9); Morinaga, 1926 (42); Grimm, 1928 (22).

Melon (*Cucumis melo*). de Candolle, 1865 (10); de Vries, 1870 (11); Haberlandt, 1875 (26); Oudemans and de Vries, 1878 (46); Harrington, 1923 (30); Kotowski, 1927 (36).

Beet. Haberlandt, 1874 (25); Oudemans and de Vries, 1878 (46); Reynolds, 1904 (47); Harrington, 1923 (30); Kotowski, 1927 (36); Bremer, 1929 (8).

Radish. Lefébure, 1801 (38); Haberlandt, 1874 (25); Coffman, 1923 (9); Kotowski, 1927 (36).

Lettuce. Uloth, 1875 (52); Coffman, 1923 (9); Kotowski, 1927 (36); Borthwick and Robbins, 1928 (7).

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RELATIONS OF GERMINATING SOY BEANS TO TEMPERATURE AND LENGTH OF INCUBATION TIME[1]

T. I. Edwards

(with six figures)

Introduction

Because much physiological study deals with plants that are secured from seeds, the conditions that prevail during the preliminary germination period constitute a very important part of such studies. Individual variability among the similar plants of an experimental series makes physiological experimentation very laborious, and presents one of the most difficult problems with respect to both technique and interpretation. Many recent experimenters have attempted to reduce variability through some sort of plant selection, and such selection usually begins with the seeds employed, —as when the seeds are chosen according to their genetic background and with reference to size, weight, color, etc. Young seedlings, just after germination, are commonly subjected to more or less rigorous selection according to size and general appearance, but especially according to apparent vigor. Such selection tends to restrict variability and furnishes experimental series of plants that are more nearly alike in their intrinsic physiological nature, as well as in their variability, than would be the case without selection. When young seedlings are selected for uniformity, the standard set of conditions that prevailed while germination was in progress becomes a basis for the selection. Consequently any advance in our knowledge of how specified lots of seed may be influenced in their germination performance by the environmental complex prevailing during the period of germination should be useful in the preparation of seedlings for experiments dealing with more advanced developmental phases. In this connection see Goodspeed (**11**), Correns (**5**), Haasis (**12**), and Tang (**36**). Furthermore, a number of students (**20**) have presented evidence leading to the

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supposition that the environmental conditions prevailing during seed germination may exert a persistent influence on the vigor and capacity for response of the resulting plant; in other words, conditions that were effective during seed germination may limit subsequent growth and yield. From these and other considerations it is evident that the subject of seed germination is an important one, not only in itself but also as fundamental to the study of more mature plants.

From the standpoint of experimental technique, studies on seed germination are in many ways much less difficult than studies dealing with more mature phases of growth and development. In many instances germination may be studied in the absence of illumination, with the avoidance of many difficult and complicated considerations that inevitably present themselves when cultures are carried out in light; for light intensity, light quality, and light fluctuation may all be left out of consideration in experiments on germination of many common sorts of seed, since ordinary seeds generally carry sufficient nutrient material to support germination and the early stages of seedling development. Although it is desirable to employ as germination medium a weak solution of inorganic salts rather than distilled water, yet excellent germination may be secured with a great variety of different solutions (**10**).

The experiments with which this paper deals show, for a specified lot of Black Eyebrow soy bean seeds, the influence of maintained temperature on the proportion of seeds germinating with incubation periods of different lengths. For a number of different combinations of temperature and time the resulting percentage values were studied by simple statistical methods, to bring out some characteristics of the variability of the lot of seed used. For each maintained temperature tested, increments of germination percentage for successive time intervals were studied.

Maintained temperature and time were the only experimental variables involved. All other influential conditions were alike for all tests, or else their complex differed from test to test only as they may have been influenced by temperature and time. Of the various influential conditions that may be varied in an experimental study, maintained temperature is especially suited to experimentation and analysis because it acts directly upon the seed protoplasm and is not subject to modification by integuments or protoplasmic membranes. For these tests temperature was an independent variable, depending only on the environment and not being significantly influenced either by the nature of the seeds or by their activity. The duration factor, time, is naturally an unavoidable component of any experimental procedure, and the length of exposure to a given set of conditions has a powerful influence on the organism's responses.

Soy bean (*Soja max* Piper or *Glycine hispida* (Moench) Maximowicz) seed was chosen as the subject of this study for several reasons. Soy bean is an important crop plant in many regions, and it has been used in a number of physiological studies as well as in field experimentation. The seeds are of convenient size for such experiments, and they germinate promptly in darkness when other conditions are suitable. A commonly cultivated, natural hybridization between different varieties of this species appears to be uncommon (**8**) and homozygous lots of seed are consequently obtainable.

A general review of the literature on temperature relations and time relations of germinating seeds is already available (**6**), and only the publications that bear directly on this study will be mentioned here. Several studies on the germination of soy bean seeds and the early growth of seedlings have been reported recently. WILSON (**40**) and OATHOUT (**28**) studied the general temperature relations of germinating soy bean seed. Employing microchemical methods, VON OHLEN (**38**) traced the major metabolic changes of early stages of development of Manchu soy beans. HAFENRICHTER (**15**) and STARK (**35**), using the same stock of seed, investigated CO_2 output and change in amino-acid content in seedlings maintained at different temperatures. JONES and TISDALE (**19**) cultured soy bean plants in pots of soil maintained at different temperatures between 12° and 40°, while the shoots were all exposed to approximately similar greenhouse conditions, the air temperature being 14°–18° for all cultures. These plants tolerated soil temperatures between 12° and 36° but failed to grow when the temperature of the soil was 40°. Root development was about the same for all soil temperatures tested above 18°. The greatest dry weight of tops was secured with soil temperatures between about 24° and about 35°. Nodule development, reckoned on the basis of dry weight, was most vigorous at a soil temperature of 24°. On the basis of JONES and TISDALE'S tests, the soy bean plant appears to be well adapted to a range of soil temperatures much broader than the range tolerated by many other crop plants that have received attention in this respect.

Experimental methods and primary numerical results

With the exception of the maintained temperatures, a uniform procedure was followed for all the experiments. The seeds lay on agar plates in covered cylindrical petri dishes 10 cm. in diameter and 1.5 cm. deep. Each experiment embraced four sets of five dishes, and each dish had 20 seeds.

The battery of maintained-temperature chambers of this laboratory (**23**), which has served for many earlier studies, was employed, but only five of the seven chambers were actually used. The automatic controls

were set so as to provide the following five temperatures: 24.5°, 28.5°, 33.0°, 36.5°, and 40.0°. The two unused chambers had temperatures of 20° and 17°. The range of temperature fluctuation for any of the used chambers was about ± 0.5°.

In this apparatus the seven chambers are in a linear series, each provided with a continuously stirred water-jacket. Each water mass is separated from the two adjacent ones by means of an uninsulated sheet-iron partition, and the entire series is inclosed in a light-proof cabinet with hair-insulated walls and bottom. Access to the chambers is had from above, by means of removable lids with cork and wood insulation. Heat is supplied at one end of the series from a thermostatically controlled tank of hot water and it is removed at the other end by means of another tank of water, the latter being thermostatically controlled by means of a mechanical refrigeration machine. The chambers are vertical cylinders (38 cm. in diameter and 38 cm. deep), and all parts of each chamber remain at practically the same temperature, but the temperature of each chamber differs from the temperature of adjacent chambers to a degree determined by the settings of the two thermostats. In each chamber the petri-dish cultures were stacked loosely, in horizontal position, in a suspended basket of perforated sheet metal.

The seeds used were all from a single lot of Black Eyebrow soy bean seed grown in Indiana in 1930, supplied by the Eastern States Farmers Exchange through the courtesy of Mr. C. W. Clemmer, of Springfield, Mass. It was received in December, 1930. These seeds were characterized by a black hilum and by a black area on each side of the hilum, the rest of the seed surface being olive green or brown. The seed-coat markings corresponded to the description given by Morse (**26**) for seeds of this variety, and the seeds of this lot were very similar to those of other lots of the same variety obtained from other sources, although those of the lot used were a little smaller on the average; 100 of them weighed 14.1 gm. There was considerable variation in the relative sizes of the areas occupied by the two colors but no obvious relations were detected between seed-coat markings and germination behavior. The whole stock of seeds was sorted at the beginning of the study and a small proportion of obviously imperfect or notably unusual individuals was discarded. The remaining supply was stored in a cloth bag in a loosely covered metal can kept in a basement room. Oathout (**28**) found such treatment very satisfactory in preserving the vitality of his soy bean seed. The experiments were performed between February 6 and March 22, 1931, so that none of the seeds used differed in age by more than a month and a half.

The medium for these tests was an agar gel containing per liter: 5.0 gm. of the Digestive Ferment Company's "Bacto-agar," 0.0018 mol. (0.2451

gm.) of KH_2PO_4, 0.00052 mol. (0.1228 gm.) of $Ca(NO_3)_2 \cdot 4H_2O$, and 0.0015 mol. (0.3707 gm.) of $MgSO_4 \cdot 7H_2O$. The salts were used because a dilute solution had been found by many workers to be more satisfactory than distilled water as a medium for seed germination. The solutes used in this way have in many instances been considered as "seed stimulants," and a great number of organic as well as inorganic substances have been assigned to this category by various investigators; NIETHAMMER (**27**) has published an extensive summary concerning seed stimulation. When seeds are germinated (and even when just preliminarily soaked) in distilled water, it is always possible that substances emanating from the seeds or from accompanying microorganisms may so modify the adjacent medium as to affect germination, and it appears that such effects are not so apt to occur when a dilute salt solution is used instead of water. Loss of essential ions from the swelling and germinating seeds appears to be retarded by the presence in the medium of suitable salts at suitable concentrations. The small traces of injurious substances that are apt to be present in distilled water seem generally to be robbed of their toxicity when nutrient salts are also present at low but considerable concentrations. GERICKE's (**10**) experiments on germinating wheat seed showed no consistent differences in the physiological effects of dilute 3-salt solutions having very different partial concentrations of the salts. His solutions were all markedly better than distilled water.

The three salts were first dissolved in distilled water from a Barnstead still, to make SHIVE's (**33**) solution R5C2 (0.175 atm.). (This low total concentration is the same as was used by GERICKE, being only one-tenth as great as the lowest total concentration used in SHIVE's series of experiments.) To 2.5 liters of the 3-salt solution thus prepared (the pH value of which was found to be 4.3), 12.5 gm. of "Bacto-agar" were added and the mixture heated in a beaker over a boiling water-bath until all granules had disappeared. Then distilled water was added to make the volume up to the original one, 2.5 liters. Each stock of gel thus prepared was stored in stoppered flasks at 2°–3°. Storage was never for more than five days, in which time no apparent alteration of the gel occurred. Its pH value was 5.6, as ascertained by the use of a quinhydrone electrode as well as by colorimetric means.

A few hours before a series of cultures was to be started, a flask of gel was removed from the refrigerator and heated in a water-bath until liquified. Then 25 ml. were pipetted into each of 20 heat-sterilized petri dishes, which were immediately placed on a previously cooled slate slab, where the agar plates rapidly solidified. This rapid cooling saved some time and lessened the amount of water that condensed on the lids of the dishes during the cooling process.

Twenty seeds, taken at random from the stock, were about equally spaced on each of the 20 plates, each seed lying on its side. The seeds sank slightly into the agar gel, and at the end of 12 hours about one-third of the seed volume was below the gel surface, the hilum being just above the surface. Thus a large area of the testa was in contact with the gel, which favored water absorption while gaseous exchange between seed and atmosphere was not greatly retarded. Rapid absorption of water took place, showing that the seed testas were readily permeable. About 25 minutes was required to distribute 400 soy beans on 20 agar plates.

It will be noted that no preliminary sterilization treatment was applied. While such treatments undoubtedly may be effective to prevent or retard subsequent development of microorganisms in the cultures, yet they are apt to exert some influence on germination. As has been remarked, a great variety of chemical substances have been observed to act as seed stimulants, and consequently it seems unlikely that any soaking treatment drastic enough to kill microbial spores would leave the seed embryo unaltered, aside from the effect of water absorption during the treatment (**9**). Preliminary soaking, which usually accompanies sterilization treatment, may in itself exert some influence on subsequent germination performance (**20**). It was found, for example, that Manchu soy beans that had received a preliminary soaking of one hour in water or in dilute solutions of ethyl alcohol required less time for germination on agar plates than was required when similar seeds had been placed on the plates without preliminary soaking. Many of these seeds were obviously injured in a single hour of soaking, however; in many instances the cotyledons were cracked or broken loose from the embryo or the testa was torn. Also the soaked seeds showed greater variability in the time required for germination than was shown by seeds laid on the agar dry. Since preliminary soaking generally hastens subsequent germination, it might be employed in studies on seed of Black Eyebrow or other varieties of soy bean if suitable time and temperature conditions were established, and if a suitable standard solution were used.

Immediately after placing the seeds, the 20 cultures of a series were installed in the requisite temperature chambers where they remained from 24 to 28 hours, except that they were removed for observation at 2-hour intervals after germinated seeds began to appear. Preliminary experiments showed when germination was to be expected at the several temperatures, and so the cultures were usually not removed for examination before the end of the tenth or twelfth hour of incubation. A culture was never out of its chamber for more than four or five minutes at any observation. The cultures were held above the level of the eye and viewed from beneath at each observation; the white radicles showed up distinctly and it was easy to decide whether a seed had germinated or not. All germinated seeds

were removed as they were found and their number was recorded. The seeds were never touched with the fingers, either in the original sorting, in placing them on the plates, or in removing or transferring them; bone-tipped forceps were always employed. The tests were discontinued before all the seeds that might have germinated had done so, because the 2-hour increments of germination percentage became small and highly variable with longer incubation periods. Satisfactory data on these later increments might have been secured only with a much larger number of repetitions than was needed to give significant values for the earlier ones.

These seeds were only rarely observed to be attacked by molds; even at the highest temperature tested such contamination was slow to appear. Whenever an ungerminated seed was seen to be attacked by mold to such an extent as was judged to threaten the contamination of neighboring seeds it was removed. Sometimes such moldy seeds were discarded but in many instances they were simply transferred to vacant depressions in the same agar plate, left by the previous removal of germinated seeds. Usually these transplanted seeds proceeded to germinate without noticeable retardation.

For this study a seed was considered to have germinated when the radicle had just broken the testa. The radicle usually grew downward within the seed coat and emerged on the lower side.

For recording germination percentages, the experimental unit was usually a set of five agar plates bearing altogether 100 seeds, 20 on each plate. The total number of seeds that had germinated in any set before any observation was consequently the germination percentage for that set and for the corresponding temperature and length of incubation period. Each test comprised four of these 100-seed sets, all incubated simultaneously at the same maintained temperature. Consequently there were four primary percentage values for each period of incubation in each test. Every test was repeated until it appeared that the range of variation of the last four primary percentage values for each length of incubation period was well within the range of variation of all corresponding values previously recorded.

There were finally available a large number of comparable primary percentages for each combination of temperature and time. The arithmetical mean and the standard error of the mean were computed for each combination, and these values appear in table I, separated by the usual $\pm$ sign. In this table the number of primary percentage values on which each mean is based is shown in parentheses below the mean. Combinations that showed no germination are indicated by dashes. The formula used by COLLINS (**4**) was employed in computing the standard error, namely,

$$\sigma_M = \sqrt{\frac{\sigma^2}{N-1}}$$

in which σ_M is the standard deviation of the mean (or the standard error), σ represents the standard deviation of individual values, and N is the number of individual values available,—in this case the number of primary percentage values for the temperature-time in question.

TABLE I

GERMINATION PERCENTAGES OF BLACK EYEBROW SOY BEANS WITH DIFFERENT COMBINATIONS OF TEMPERATURE AND INCUBATION TIME

INCUBATION TIME	24.5°	28.5°	33.0°	36.5°	40.0°
hr.					
12	---	0	3.0 ± 0.4 (12)	1.3 ± 0.4 (12)	---
14	---	3.4 ± 0.2 (8)	16.8 ± 0.6 (20)	13.5 ± 0.9 (20)	---
16	---	17.4 ± 2.2 (16)	39.9 ± 0.5 (24)	36.4 ± 1.2 (24)	---
18	6.5 ± 0.3 (12)	38.0 ± 2.0 (16)	55.6 ± 0.9 (20)	56.5 ± 1.2 (37)	3.8 (2)
20	21.6 ± 2.1 (12)	52.6 ± 1.7 (12)	70.3 ± 1.3 (12)	69.4 ± 0.8 (28)	15.0 ± 0.1 (10)
22	37.4 ± 2.2 (12)	67.8 ± 1.9 (11)	76.8 ± 1.5 (8)	76.3 ± 1.2 (21)	39.3 ± 2.2 (10)
24	51.6 ± 2.2 (12)	77.0 ± 1.6 (7)	80.8 ± 1.4 (8)	79.4 ± 1.2 (13)	58.6 ± 1.6 (10)
26	63.4 ± 0.9 (12)	---	---	84.4 ± 1.6 (12)	70.0 ± 1.6 (8)
28	70.6 (4)	---	---	---	72.4 (4)

As might be expected, there was considerable variation in each series of primary percentage values. The characteristics of the variation usually encountered are shown by the following representative frequency tabulation for the combination of 18 hours and 36.5°. (In the preparation of this tabulation the experimental unit was a single agar plate, instead of five plates, and there were 183 comparable primary values. The largest possible value is of course 20 instead of 100.)

Seeds germinated:	5	6	7	8	9	10	11	12	13	14	15	16	17
Frequency:	0	5	9	8	19	26	34	26	22	21	10	3	0

For this series the average number of germinated seeds is 11.1 and the value for the crude mode is 11.0.

These data are presented graphically by the frequency polygon of figure 1, where the possible values for the number of germinated seeds per plate are plotted on the horizontal axis and the frequency with which each

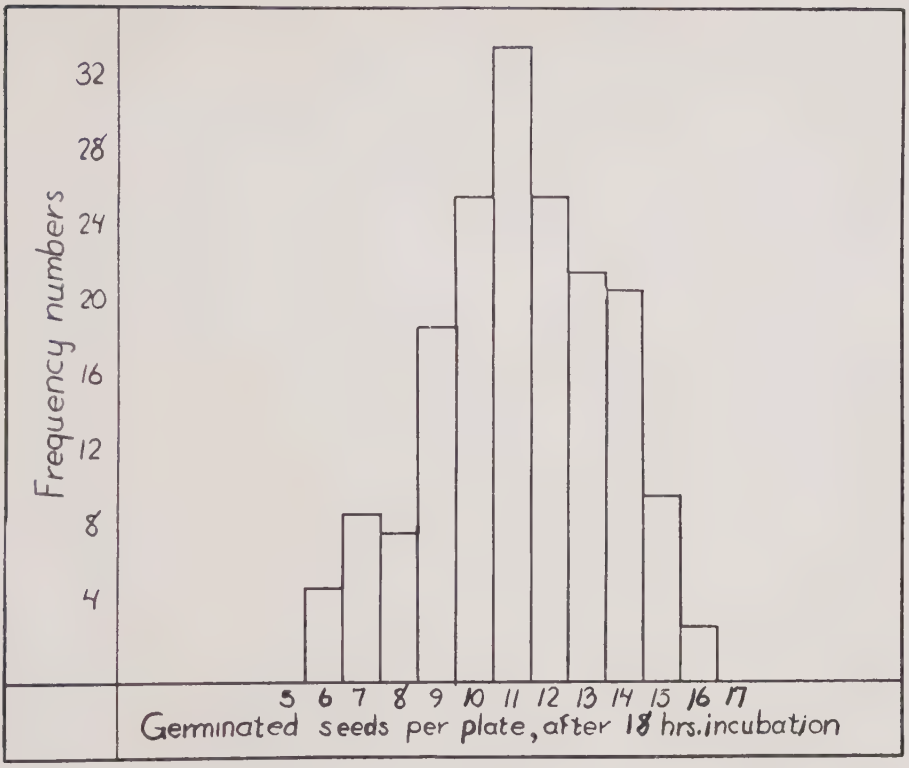

FIG. 1. Frequency polygon showing number of times that various numbers of Black Eyebrow soy bean seeds were found germinated after 18 hours of incubation at 36.5° (based on data from 183 plate cultures, each with 20 seeds).

value occurred is plotted as the height of a rectangle. This polygon indicates that a large proportion of the individual values falls close to the average value (11.1) and in approximately equal numbers on either side of it, and also that any percentage value occurs less frequently as its magnitude is farther removed from that of the average. In these general respects this polygon is similar to the curve representing the theoretical distribution of errors. These observations, and similar ones derived from tests with other temperature-time combinations, appear to justify the use of the averages, each with its standard error, as indices of the general performance of the lot of seeds with which this study deals.

Two sets of criteria might be used for judging the extent of the deviation of the individual values: (1) frequency polygons (such as that of figure 1) constructed from the values used in the computation of the means; and (2) the standard deviation of the means, which are statistical constants. The polygon of figure 1 may serve as a representative sample in detail, but

the tendency for the values of any comparable series to fall close to their arithmetical mean, and hence the relative significance of the latter in representing the whole series, is indicated by the standard error of the mean.

As has been mentioned, each culture was temporarily removed for a short time from its temperature chamber whenever an observation was made; *i.e.*, at 2-hour intervals after the lapse of 10 or 12 hours. All the petri dishes of a set were removed from the chamber together and were returned together after observation. The greenhouse room in which the temperature chambers are, and in which the cultures were examined, had an air temperature usually between 20° and 25° C., and each culture naturally tended to assume the room temperature, becoming either warmer or cooler, while it was out of its chamber. Also, while outside of their chambers the petri dishes and their contents were illuminated, although they were in darkness throughout most of the experimental period. It is conceivable that these short temporary interruptions in the otherwise even tenure of the maintained conditions of temperature and lack of illumination for any culture might exert some sensible influence on germination.

To determine whether this was really so, four special experiments were carried out with a temperature of 36.5°. In each of these experiments there were 25 dishes, 10 of which were removed, examined, and returned to the chamber in the usual way, at the end of 12, 14, 16, and 18 hours while the remaining 15 were not removed and examined until after 18 hours of incubation. For the 18-hour period the average germination percentage was 56.3 ± 1.20 for the first group (which had been subjected to temporary fluctuation) and the corresponding average for the second group was 54.5 ± 1.19. Since the difference between these values cannot be regarded as significant, it may be concluded that the environment changes in question did not alter the course of germination appreciably.

Discussion

General survey of results

Some of the general relations of the average germination percentages for the stock of Black Eyebrow soy bean seed used in the experiments are readily visualized when the averages of table I are plotted as graphs. A simple and direct graphic presentation of these data is shown in figure 2, which embraces seven separate temperature graphs, one for each of the different lengths of incubation period employed. Abscissae are maintained temperatures and ordinates are average germination percentages. Each graph represents the temperature relations of seedling production by this lot of seed in the specified time period. They appear like a group of slightly asymmetrical, downwardly concave curves, with a common maxi-

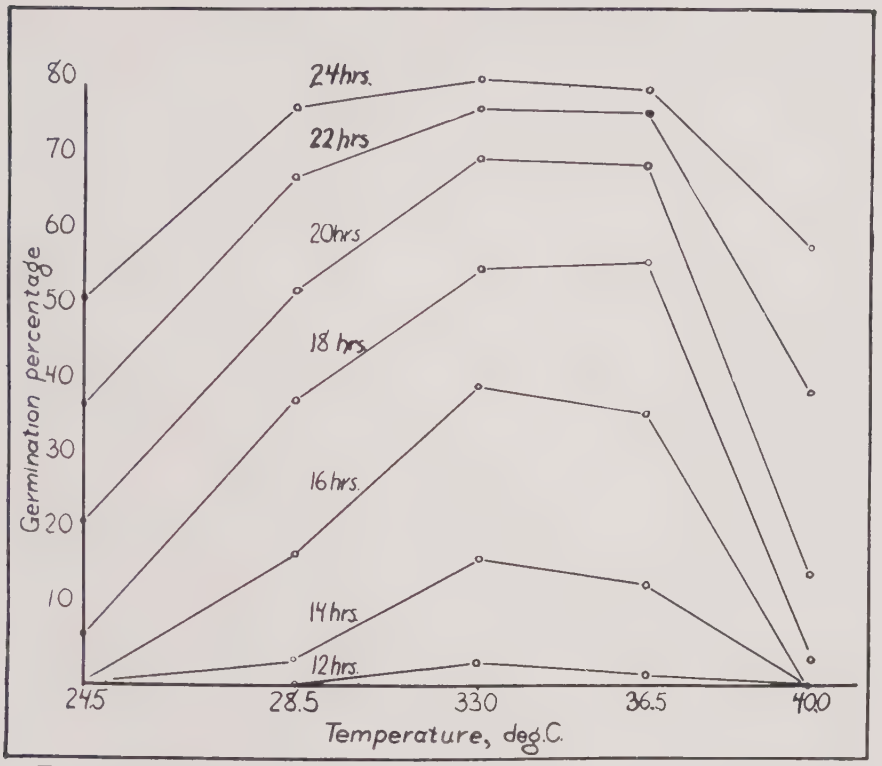

FIG. 2. Temperature-germination graphs for Black Eyebrow soy bean seed, for several lengths of incubation period. Each period length is represented by a separate graph.

mum in the region representing a temperature range from about 33.0° to about 36.5°. Each curve has a steeper slope on the high-temperature side than on the low-temperature side of its peak. For each different length of tested incubation period the temperatures 33.0° and 36.5° were in close agreement with respect to their average germination percentages, but of course the averages are greater as the period was longer. The three other temperatures all gave lower percentages. Cultures at 28.5° gave somewhat lower germination percentages than cultures of the same age at 33.0° or at 36.5°, but those at 28.5° always gave values higher than the corresponding ones at 24.5° or 40.0°. The optimal temperature range for each of the different period lengths consequently extends from about 33.0° to about 36.5°. No shifting of this optimal temperature range with time, such as has been described by several experimenters with other kinds of seed and with various experimental methods, is apparent here.

Successively longer incubation periods broadened the temperature range within which germination was observed. For the 12-hour, 14-hour, and

16-hour periods, minimal and maximal temperatures for the occurrence of germination are apparent, since in these instances two or more of the tested temperatures failed to give any germination. For the longer periods, however, the minimal and maximal temperature values clearly fall outside the temperature range embraced by the tests. With increasing length of period the graphs become progressively flattened at the top and the several tested temperatures tend to approach equal effectiveness with respect to seedling production. WILSON (**40**) found that approximately the same percentage of soy bean seeds eventually germinated at each of the temperatures he tested between 10° and 30°.

In connection with the present study, some experiments with seed of the Manchu, Minsoy, and Tokyo varieties of soy bean showed optimal temperature ranges with upper limits below that of the optimal range for the Black Eyebrow variety as here shown; in most of these additional instances 33.0° was more favorable for germination than either 36.5° or 28.5°.

In addition to the results thus far mentioned, further examination of the data of table I brings out a number of less obvious relations, some of which are considered in the following sections of this paper. It is to be borne in mind that these results and statistical relations apply strictly only to the experiments of this study, to the particular stock of Black Eyebrow seed used, and to the specified conditions and technique of this experimentation. Time factors are of course approximated only in terms of the 2-hour observation intervals, and all critical temperatures, as well as the lower and upper limits of critical temperature ranges, are naturally to be regarded as ranges of temperature rather than as definite temperatures. With more laborious and more time-consuming experimental procedure, and with still further repetitions of the tests, these approximations might have been given a higher degree of precision, of course; but the numerical results appear to be sufficiently precise to furnish some additions to our knowledge of the performance of samples of germinating seeds. The following discussion will be in terms of temperature ranges rather than in terms of exact temperatures, and each critical temperature mentioned may be considered as approximate to within a few degrees. The need for brevity of statement and for general clearness frequently limits the extent to which considerations concerning relative degrees of precision may be mentioned throughout reports on many biological studies. It is interesting to note that the inevitably approximate nature of critical temperatures for seed germination was clearly emphasized by HABERLANDT (**13**) as early as 1874, who wrote:

"Denn die Temperaturgrenzen bei welchen eine Schwächung und Verlangsamung des Keimungsprozesses eintritt, ist keiner scharf gezogene

Linie, sondern einem Streifen zu vergleichen, der unter Umständen eine Verschiebung der Grenzlinie für den Samen einer bestimmten Qualität wohl gestattet. Die mehr oder weniger vollkommene Reife und Ausbildung des Kornes, sein Alter, seine Heimath u. s. w. werden darauf Einfluss nehmen können, daher man mit der Angabe sich wird zufrieden stellen müssen, dass für irgend eine beliebige Samenart die obere Grenze zwischen diesen und jenen Graden schwankte und entweder näher der einen oder der anderen Zahl liege."

Course of germination at maintained temperatures

Although it is interesting to note that the graphs of figure 2, which represent the performance of a population of seeds subdivided into cultures that were incubated at different temperatures, bear a superficial resemblance to many temperature graphs of physiological processes, such as growth, yet it must be remembered that the germination percentages of this study do not refer to process rates in individual seeds. The percentages are statistical values representing the various proportions of a seed population that were able to attain a specified developmental stage in cultures of certain ages at the specified temperatures. These proportions are dependent, not only upon prevailing environmental conditions and the duration of incubation, but also upon differences in physiological behavior among the individual seeds constituting the population. The percentage values provide a means for analyzing the physiological variability of the individual seeds within the population and they will now be considered with reference to several types of analysis designed to reveal some features of the nature and extent of this diversity.

In the graphs of figure 3, germination percentages are represented as ordinates and lengths of incubation time are abscissae, the data for each temperature being plotted separately. The form of each graph shows how the original seed population became gradually transformed, with lapse of time, into a population of seedlings. For all five tested temperatures this transformation evidently followed the same general course. All five graphs are sigmoid and their respective apparent points of inflection are indicated by circles. In general, the cumulative germination percentage increased slowly during the earlier observation periods, and then more rapidly, until a percentage corresponding to the apparent point of inflection of the graph was reached, after which the rate of increase fell off more and more. The five graphs are so similar that if they were plotted as arising from a common abscissa they would nearly coincide throughout. One feature of their close similarity is well shown by the fact that the percentage values for their apparent points of inflection all fall within the narrow range from about 36.4 to about 39.9.

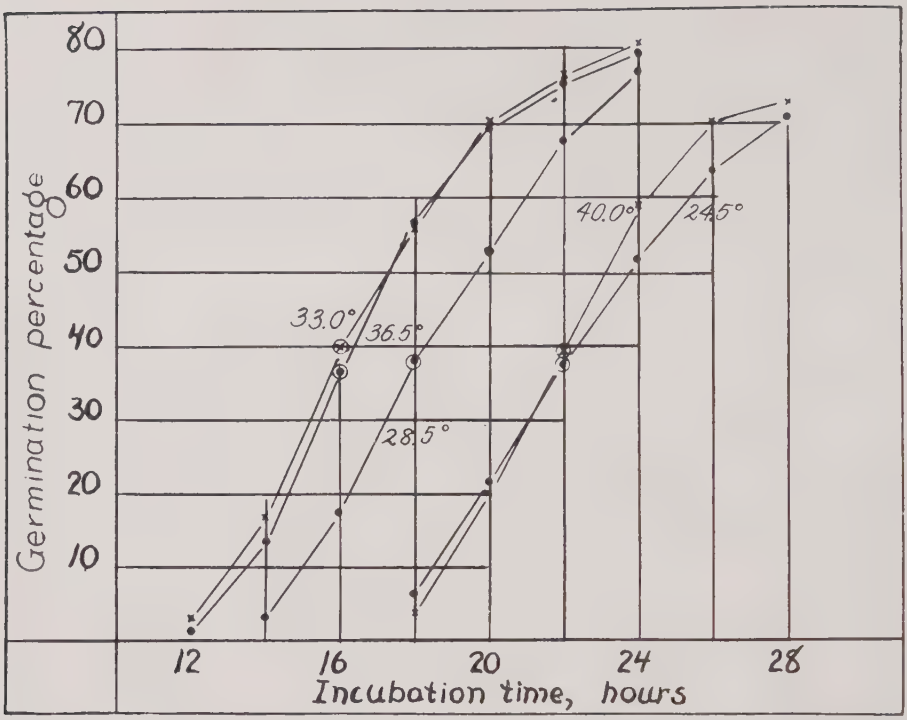

FIG. 3. Graphs for Black Eyebrow soy bean seed showing germination-percentage values as related to incubation time. Each graph represents a different temperature. Approximate points of inflection shown by circles.

The principal differences among these graphs are with respect to the time periods they represent. The graphs for 33.0° and 36.5° are seen to be almost identical in this regard and those for 24.5° and 40.0° are not greatly divergent, but the graph for 28.5° stands by itself. Less than 12 hours of incubation at 33.0° or 36.5° sufficed for the appearance of the first seedlings; in cultures at 28.5° the first seedlings were observed only after 14 hours of incubation; and seedlings did not begin to appear in cultures at 24.5° or 40.0° until these had been incubated for 18 hours. This same relationship between incubation time and temperature persisted throughout this study; any given percentage value was attained in cultures at 33.0° or 36.5° about two hours earlier than in cultures at 28.5°, and about six hours earlier than in cultures at 24.5° or 40.0°.

This kind of graph, showing progress of seed germination as related to lapse of time, has not been employed by many writers in this general field; but many papers give tabular data from which graphs of this sort may be constructed, and such graphs would generally be similar in their essentials to those shown in figure 3. Among the publications in which special

attention is given to this kind of analysis may be mentioned HARRINGTON'S (**16**, p. 348) germination-time graphs for his lot of seed of *Impatiens balsamina,* which present important relations and do not differ in general form from those of figure 3. The graphs given by EVANS (**7**), derived from seed of *Amaranthus retroflexus* germinated at nine different temperatures, show more variability in form than those of figure 3. A temperature of 42° gave the highest time rate of seedling production. The first seedlings appeared later in cultures at 46.1° than in those at 42°, and the cumulative increase in germination percentage was slower in cultures at 46.1°.

INCUBATION TIMES CORRESPONDING TO REPRESENTATIVE PERCENTAGE VALUES

As an alternative to representing the data of table I in terms of germination percentages attained after incubation periods of definite length, it is possible by interpolation to obtain from these data the lengths of time that correspond to certain definite germination percentages. The results of such a series of interpolations are shown by the graphs in figure 4, in which lengths of incubation time are measured on the vertical axis while temperature is indicated on the horizontal axis. There are three graphs, for 3, 38, and 70 per cent. respectively. These particular values are selected for special study because mean percentages of about these magnitudes appear for several temperatures in the table, and because the percentage value 38 is especially interesting since it represents the proportion of seeds that had germinated before the rate of seedling production began to fall off (see figure 3, the points of inflection). The graphs of figure 4 are concave upward, the low-temperature ends of the ones for 3 and for 70 per cent. being farther apart than the high-temperature ends. This difference between the lengths of time corresponding to these two percentages decreases as higher temperatures are examined, and it is interesting to note that there is no evident alteration of this relation as the optimal range of temperatures is approached and passed. These graphs suggest that an optimal range of temperature for seed germination might be taken as the range for which the least time is required for the germination of a specified proportion of the lot of seed studied. Thus the temperature range 33.0°–36.5° might be considered as optimal for the percentage range 3–70, according to the graphs of figure 4.

These graphs may be viewed as contour lines on a 3-dimensional diagram showing the relations of temperature, incubation time, and germination percentage. For each germination percentage many different lengths of incubation period are each seen to have two separate temperature ranges equally favorable for seedling production, one of these being suboptimal and the other supraoptimal.

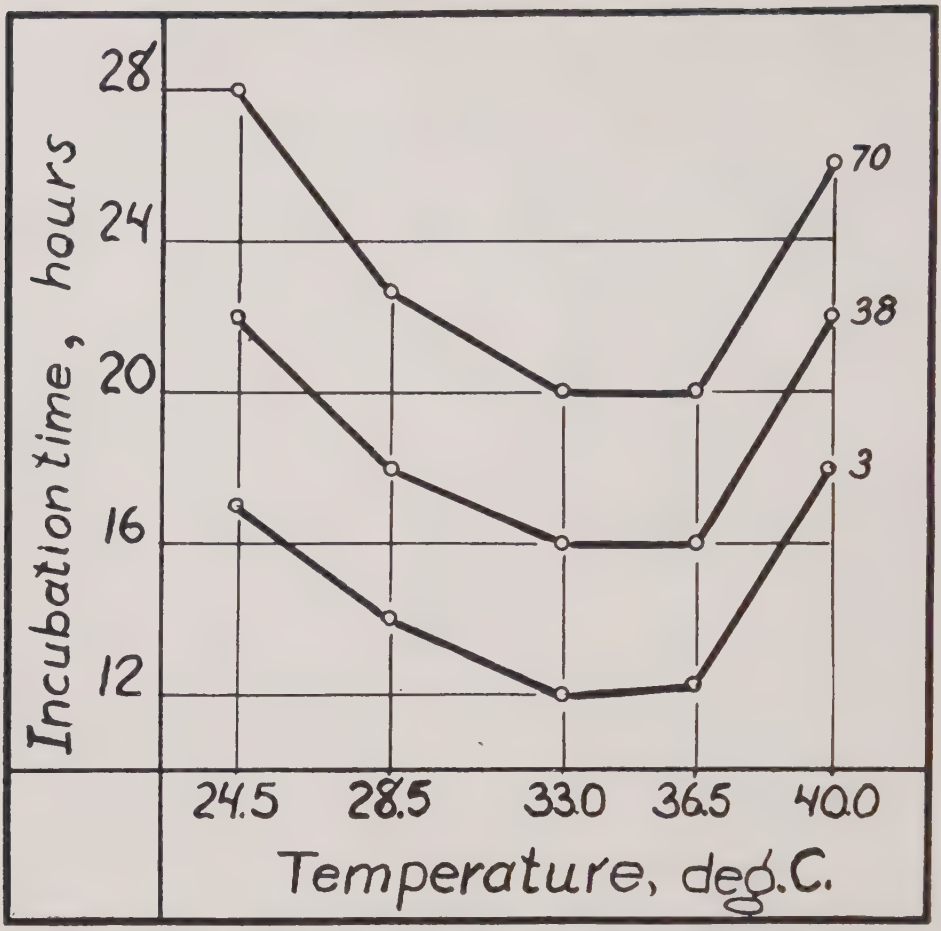

FIG. 4. Graphs showing relation of temperature to length of incubation time required for germination of specified percentages of Black Eyebrow soy bean seed. Each graph represents a different germination percentage.

Similar relations of incubation time to temperature have been reported by several workers. In the course of experiments on temperature relations of nuclear phenomena, HARTMANN (**17**) made some preliminary germination tests at eight constant temperatures between 8.5° and 41.0°, using moist sawdust as substratum. For seed of *Phaseolus multiflorus, Helianthus annuus,* maize, and pea, 31° induced the earliest appearance of seedlings; at higher and lower temperatures seedlings appeared later. BREMER'S (**3**) results for germinating beet seed show an optimal temperature range (22°–36°) for which the production of seedlings required only one or two days of incubation; with higher and lower temperatures longer

incubation was necessary. Reynolds (**32**), Kotowski (**21**), Wilson and Hottes (**41**), and Wilson (**40**) noted a diminution in the incubation period necessary for germination as higher temperatures were examined, but these tests, which embraced no temperatures above 30°–32°, did not involve temperatures high enough to induce retardation of germination. Haberlandt (**13, 14**), Kreysing (**22**), and Bär (**2**) also found definite evidence of a retardation in seedling production at high temperatures as compared with lower ones. Livingston and Haasis's (**24**) graphs showing the relations of temperature, incubation period, and germination percentage for their French rice seed are similar to the graphs of figure 4, but the range of period length between the beginning of germination and the attainment of 90 per cent. is shown to be narrower for the optimal temperature (about 34°) than for suboptimal or supraoptimal temperatures.

Increments of germination percentage

It is evident that the seeds tested in this study varied considerably in rate of response to uniform environmental conditions, because if all the seeds had possessed identical physiological properties every radicle should have emerged after the same period of incubation at any given temperature. Although indications as to the nature of the diversity existing within a seed population may be obtained by inspection of sets of graphs such as those of figure 3, some features of this diversity may be more readily appreciated if the increments of germination percentage from observation to observation are employed. Such percentage increments, computed from the data of table I, are set forth by means of the histograms of figure 5, in which the 2-hour observation intervals of the incubation period are indicated on the horizontal axis while the increments in question are plotted vertically, each of their values being inscribed just above its horizontal segment of the graph. This set of graphs thus constitutes a table of values arranged in graphic form. Each of the five temperatures is represented by a separate graph.

Increments of germination percentage may be treated in either of two ways. (1) Attention may be focused on the fluctuating rate at which the original seed population is transformed into a seedling population and the average rates for the several 2-hour intervals may be compared to bring out acceleration and retardation. Since counts of germinated seeds were made at each observation, these rates for the several intervals are to be read directly (as percentages of the seed population) from the graphs of figure 5. Or (2) the percentage increments may be considered as measures of one kind of physiological diversity which was latent in the seed population. The histograms of figure 5 thus become frequency polygons that show

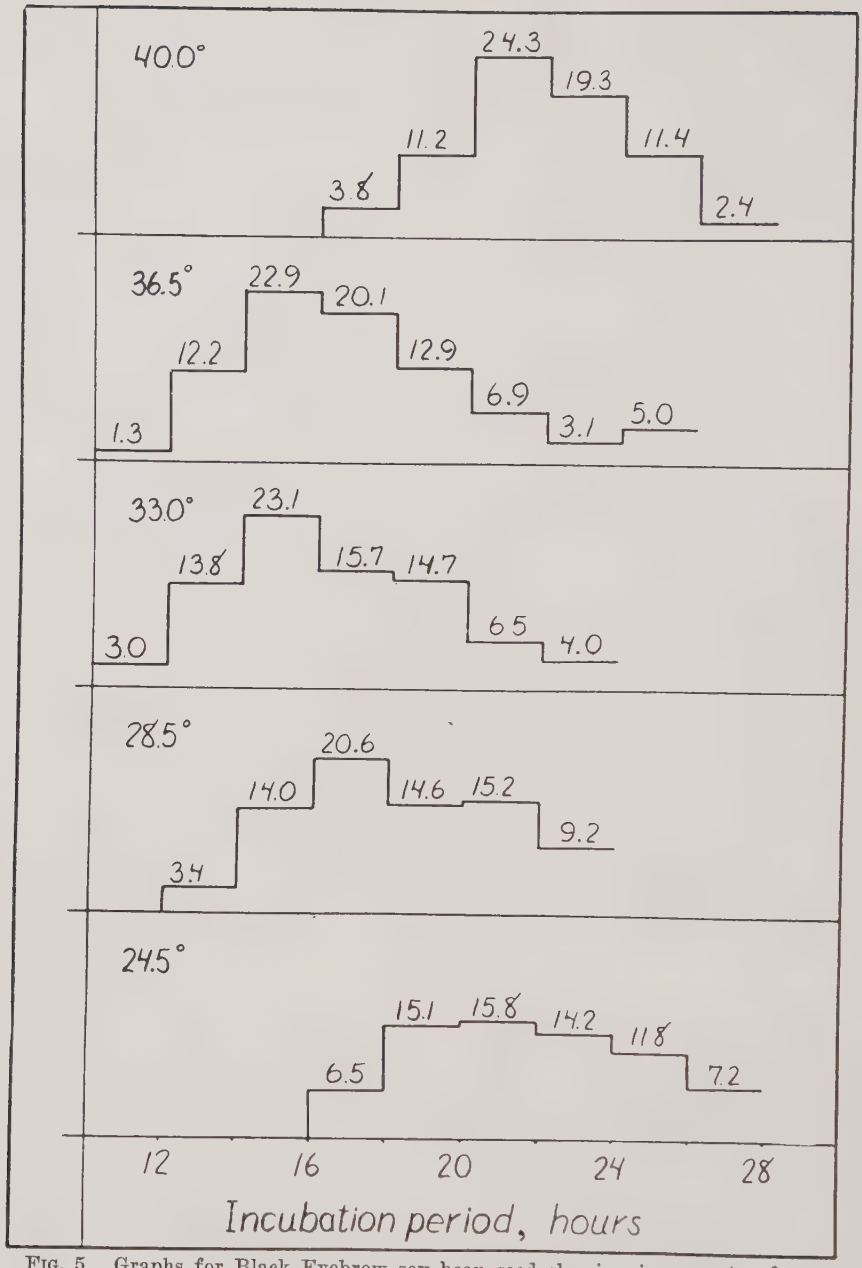

FIG. 5. Graphs for Black Eyebrow soy bean seed showing increments of germination percentage for 2-hour intervals. Each graph represents a different maintained temperature.

the relative proportions of the population that were embraced in several physiological classes of seeds. Since both these modes of treatment are instructive, the 2-hourly increments of germination percentage will be discussed in terms of each, in turn.

Rates of seedling production.—The rate of seedling production is seen (fig. 5) to have changed in a rather regular way for each of the five temperatures tested. Beginning with a low value for the first interval that yielded any seedlings at all, it increased rapidly to a maximum, shown for the third 2-hour interval of seedling production, and then it decreased. For the three lower temperatures the rate for the fourth interval is about like that for the second, and for the two higher temperatures this is true of the rate for the fifth interval. For subsequent intervals the rate generally continued to decrease progressively. The decrease is usually seen to have been less rapid than the earlier increase; *i.e.*, retardation of the rate of seedling production was less rapid than acceleration. The differences between successive values of the rate are more marked for the higher temperatures than for the lower ones; for 24.5° there is relatively little difference between the 18th hour of incubation and the 26th hour, and no doubt this tendency toward uniformity of rate throughout many consecutive 2-hour intervals would be still more pronounced if still lower temperatures had been employed. There was evidently a direct relation between the incubation temperature and the maximum rate of seedling production, the rate for the third interval after seedlings began to appear being greater as the temperature was higher.

Since the peaks of the graphs of figure 5 mark the respective points at which seedling production began to fall off, the abscissa of the peak for each temperature is equivalent to the abscissa of the apparent point of inflection on the corresponding graph of figure 3. Both figures consequently show the same relations between incubation time and the maximal rate of seedling production for each tested temperature, for the rate of seedling production is of course just the rate of increase in the cumulative germination percentage in each series. Table II shows these relations.

Although the maximal 2-hour rate of seedling production and the duration of incubation required for the development of that maximal rate are both seen to have varied considerably and consistently with the degree of temperature employed, yet the total germination percentage corresponding to the apparent points of inflection on the graphs of figure 3 (and hence to the maximal rates of seedling production) appears to have shown no significant and consistent variation with temperature. This apparently critical percentage value is constant within the narrow range from 36.4 to 39.9, and its average magnitude for all five temperatures is 38.2. This is a remarkable constancy, and it is at once suggested that we have here a critical

TABLE II

MAXIMAL 2-HOUR RATES OF SEEDLING PRODUCTION AND CORRESPONDING GERMINATION PERCENTAGES AND LENGTHS OF INCUBATION PERIOD AT DIFFERENT TEMPERATURES DERIVED FROM FIGURES 3 AND 5

MAINTAINED TEMPERATURE	MAXIMAL 2-HOUR RATE OF SEEDLING PRODUCTION (FROM FIG. 5)	GERMINATION PERCENTAGE CORRESPONDING TO MAXIMAL RATE OF INCREASE IN GERMINATION PERCENTAGE (FROM FIG. 3)	INCUBATION PERIOD
°C.	%	%	hr.
24.5	15.8	37.4 ± 2.2	22
28.5	20.6	38.0 ± 2.0	18
33.0	23.1	39.9 ± 0.5	16
36.5	22.9	36.4 ± 1.2	16
40.0	24.3	39.3 ± 2.2	22

index of some characteristic or combination of characteristics of this lot of seeds. Apparently about 35–40 per cent. of the individuals were able to germinate with exceptional promptness, no matter which of the five widely different temperatures was employed. Whether the group of specially prompt and highly temperature-tolerant seeds (with respect to their capacity for germination) would embrace the same individuals for all favorable test temperatures is of course not known, for this apparently significant percentage is merely a statistical value pertaining to the entire lot.

Such an index may perhaps be of value in practical seed testing, for useful viability indices of stocks of seed may well refer definitely to capacity for prompt (although incomplete) germination of representative samples under a variety of environmental conditions, rather than to capacity for ultimate (and more nearly complete) germination of the samples with an environmental complex that is specially favorable to germination without reference to promptness. Several investigators mentioned by TOUMEY (**37**, p. 128) have recognized that the percentage of seeds germinating before the rate of seedling production begins to fall off may be used to represent the capacity of a given lot of seeds to produce seedlings quickly. In many situations encountered in forestry and agriculture, plant competition is so severe that an early start may be essential if a plant is to survive. TOUMEY used the name "germinative energy" for this percentage value, a term not wholly satisfactory, since ambiguity in the broader usages of science is apt to arise when a word (such as energy) with a definite meaning in one field (physics) is used in a different sense in another field (biology).

PHYSIOLOGICAL CLASSES OF SEEDS.—If attention be directed to the time spans covered by the process of extrusion of radicles, the degree of diver-

sity of individual seeds with respect to rate of response to the prevailing germination conditions becomes more apparent. For instance, the first seedlings were found after 12 hours of incubation at 33.0°, and after 24 hours only 80 per cent. of the population had germinated. It seems evident that there must have been important physiological differences of some kind among the seeds of this lot, when, for example, some seeds required only 12 hours and others required 24 hours to reach the same stage of development, although all were treated alike.

Since the cultures were examined every two hours and all germinated seeds were removed at each examination, this procedure operated as a sorting process, the seeds falling into one or another of several different physiological classes. Each class was characterized by rates of response of its members to specified conditions of incubation. If the histograms of figure 5 are examined with this concept in mind, then the intervals on the horizontal axis represent the various physiological classes into which the seeds were sorted, and the height of each segment or block of the histogram indicates the relative size of its particular class. Comparison of the five histograms brings out some notable similarities. Each shows a tendency for the values for length of incubation time to fall close to a crude mode (the third interval measured from the beginning of the appearance of seedlings), and the farther removed a class is from the crude mode the fewer are the individuals in it. Furthermore, the number of individuals with longer incubation periods than the modal length exceeds the number with shorter incubation periods, and each graph is consequently somewhat asymmetrical. In these respects the histograms are not unlike many other frequency polygons representing the distribution of some quantitative character among the members of a population of organisms. In connection with the study here reported, similar data were obtained for several additional kinds of seed, including three soy bean varieties and *Alisma plantago-aquatica* L.; in nearly all instances where large seed samples were employed with a maintained temperature, a frequency polygon essentially much like those shown in figure 5 could be constructed from the experimental results. Perhaps the most remarkable feature of these histograms representing a grouping of the seeds into physiological classes is their evident similarity with respect to the size relations of the several classes. That this sort of graph takes so nearly the same form for all five temperatures renders this relation much more striking and apparently more significant than it might be if all tests had been carried out at the same temperature.

It will be observed that the higher-temperature graphs of figure 5 exhibit more abrupt steps or gradations from class to class than are shown by the lower-temperature graphs. This is probably related to the fact that the processes of germination went forward more rapidly at higher tempera-

tures than at lower ones. Obviously the results of this kind of sorting should depend not only on the statistical distribution of the different kinds of individuals in the original seed sample, but also on the time-temperature characteristics chosen for the sorting procedure. For example, if it were required to sort 80 per cent. of the seeds of a sample into a specified number of classes, and if the relative sizes of the several classes were also specified, then this sorting technique might be applied by using any one of a number of different maintained temperatures, but the observation intervals would need to be shorter for higher temperatures than for lower ones.

Probably the properties of the agar gel on which the seeds rested changed during incubation, and the slowly developing seeds may not have been subjected to exactly the same environmental conditions as the rapidly developing ones. If it had been possible to maintain the environmental conditions constant throughout the period of incubation, perhaps the rates of seedling production might have been somewhat different. If greater changes in the medium took place at high temperatures than at low ones, some of the differences in form of the graphs of figure 5 might be accounted for. Such considerations as these, however, cannot alter the significance of the fact that the rates of response of the individual seeds were not alike, for all the seeds of any culture received similar treatment at the start.

Although many students of seed germination have been aware that counts made at intervals in a germination experiment furnish data resolvable into graphs similar to those of figure 5, nevertheless this aspect of the general problem of germination relations has rarely been discussed. Perhaps one reason for this may be that many experimental studies on seed germination have been made with relatively small seed samples. With small samples the increments of germination percentage are naturally also small, and they are likely to be irregular as well. In the present study the seed samples were relatively large (from 800 to 3600 individual seeds per sample), and this seems to have insured for every sample a fair representation of all the physiological classes in the stock, smoothing out many irregularities of the component tests,—irregularities that might have obscured important relations if the samples had been much smaller.

Earlier studies on physiological classes of seeds in same stock.—Goodspeed (**11**) made daily germination counts on lots of seed from a great number of plants in his breeding experiments on tobacco, and showed how the daily percentage-increment values altered throughout a long experiment period. Recognizing that the regular removal of germinated seeds from the cultures results in a physiological classification, Goodspeed proposed this as a method for analyzing the genetic constitution of the progeny of hybrid plants. His results refer to seed samples that were not very large (100–200 seeds per sample), and some of the irregularity in the relative magnitudes

of his increments may have been due to inadequate sampling. Expressed in the form of frequency polygons, his data take a variety of forms; most of the graphs show only one mode, but those for some samples of seeds from hybrid parents show two modes. A few are symmetrical and there is no apparent agreement among the others with regard to relative steepness on the two sides of the mode.

Although not concerned with seed germination, LOEB and NORTHROP'S (25) data on the temperature relations of the development of fruit flies (*Drosophila*) are of interest here. Flies were grown from eggs under controlled conditions at several constant temperatures between 10° and 31.5°. At regular intervals each culture was examined and record was made of the number of larvae present. Similar observations were subsequently made on pupae and on the emergence of imagoes. For each stage of development the same kind of individual variation was noted; the rate of appearance of individuals at any given developmental stage rose to a maximum and then fell off, usually more slowly than it had risen. Figure 6 shows the temperature-time relations of the emergence of *Drosophila* imagoes, the daily emergence-percentage increments for which have been computed from LOEB and NORTHROP'S tables. These histograms are plotted like those of figure 5 and require no special explanation. Rates of emergence are an expression of individual variation in capacity for development, and a study of this kind shows not only the constitution of the population under consideration but also the extent to which the units of the population are influenced by the various sets of conditions employed in the experimentation.

WAGGONER (39) studied the progress of germination in samples of radish seeds with various water contents, the samples having been subjected to a temperature of 80° for 30 minutes before being distributed to germinate on wet plaster-of-Paris blocks. Samples with original water content of 18 per cent. failed to show germination, and those with 14 or with 9 per cent. moisture showed limitation and retardation of germination as compared with the sample having water content of only 4 per cent. Daily counts of germinated seeds showed clearly the manner in which the time rate of seedling production increased to a maximum and then decreased. Histograms constructed from WAGGONER'S tables possess the same general characteristics as those shown in figure 5.

This germination method of separating physiological classes in a stock of seed has been used to advantage by CORRENS (5). Seeds from artificially pollinated plants of *Melandrium* (a dioecious form) were incubated on moist paper and the seedlings transplanted to soil as they appeared, being classified into quartiles on the basis of the time required for their emergence from the seed. Growing 11,558 of these seedlings to a developmental stage

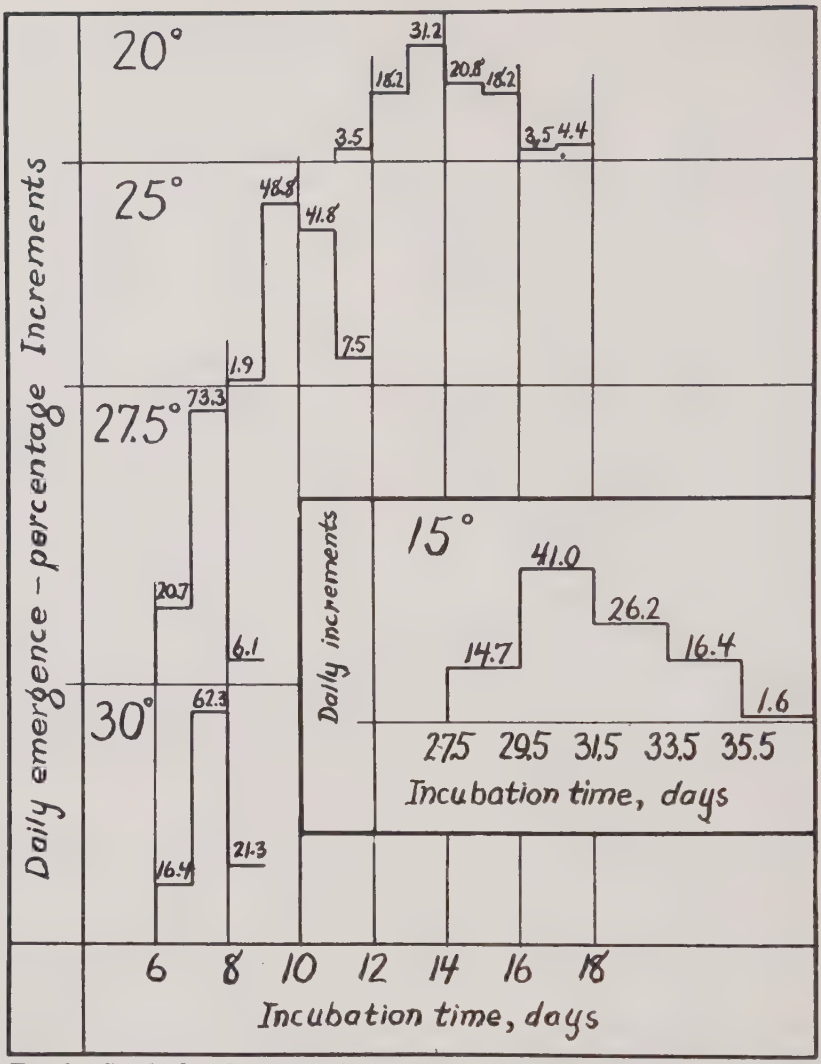

FIG. 6. Graphs based on data from LOEB and NORTHROP, showing rates of emergence of *Drosophila* imagoes at several maintained temperatures.

that permitted the staminate and pistillate individuals to be separately counted, CORRENS found that seeds destined to produce staminate plants tended to germinate more promptly than the others. In the progeny of one staminate parent the percentage of pistillate individuals in the first quartile (most rapid germination) was 32.8; in the second, 52.5; in the third, 62.6; and in the fourth (representing the seeds that had germinated most slowly), 69.0. Incidentally he observed that the rate of emergence

of seedlings rose to a maximum on the second or third day of incubation and declined thereafter.

PIEPER (**30**) observed that there was no correlation between the rate of germination of parents and of progeny in several samples of *Poa pratensis* seed from which he raised plants and obtained seed. He noted, however, that seeds from slowly germinating parents tended to germinate slowly, and that plants from rapidly germinating seeds produced seeds that tended to germinate rapidly.

PEARL (**28a**) observed differences in growth rate among seedlings sorted into physiological classes by methods similar to those used in the present study. After preliminary soaking, *Cucumis melo* seeds were sown on agar plates, incubated in darkness at 30°, and observed several times during the period of emergence of radicles. At each observation all germinated seeds were transferred to fresh agar plates and allowed to grow for an additional 48 hours. At the end of this growth period each group of seedlings was divided into cotyledons, hypocotyl, and roots, measurements of moist and fresh weight being made. The combined weight of hypocotyl and roots was taken as a measurement of growth. He found that the seeds which took the longest time in germinating made the least growth subsequently, and the best growth was made by the classes of seeds that germinated rapidly.

Both HAASIS (**12**) and TANG (**36**) called attention briefly to the possibilities of this sort of physiological technique for the sorting of a sample of seed into classes, but they gave only very limited attention to the question concerning the physiological classes of seedlings. The possibility of employing the temperature-time relations of seed germination as criteria in selecting similar lots of seedlings for physiological experimentation has been emphasized by LIVINGSTON and HAASIS (**24**).

Variation in the physiological characteristics of seeds may be manifested in other ways besides considering rates of seedling production. PORODKO (**31**) contributed information concerning the death rates at high temperatures, and SIFTON (**34**) reported on the death rates of seeds during storage. For each of these sets of data the decrement of the germination percentage may be studied and graphs similar to those of figure 5 may be constructed which bring out interesting relations. If, like HEWLETT (**18**), one suitably applies a toxic substance to a seed population for a given time period some of the seeds die, and these constitute a class whose individuals must have been characterized by about the same degree of resistance; if a suitably longer exposure period is used the seeds of still another class are killed, and so on. Naturally, if seeds must be killed in order to study their physiological variation, this method may be regarded as of limited usefulness in some respects; but the results secured by its application provide additional means

for comparing different lots of seed with respect to their intrinsic physiological variability.

Mean length of incubation time

Haberlandt (**14**), Pieper (**29**), Akemine (**1**), Gassner (**9**), and Kotowski (**21**) expressed the time relations of the germination of their seed samples by means of a mathematical expression or index that has considerable promise of usefulness when different lots of seed are to be compared. This expression is the mean length of incubation time required by the seed sample in question, for germination under specified test conditions. Kotowski used the reciprocal of the mean length of incubation time, calling it the "coefficient of velocity" of germination.

To derive the mean length of incubation time, a seed sample is tested in the manner employed in the present study, with frequent observations and records of the germination-percentage increments for the several observation intervals. Then each percentage increment is multiplied by the corresponding length of incubation period, measured from the beginning of the test, and all resulting products are summed. Finally, the sum thus secured is divided by the total germination percentage for the whole test; that is, by the sum of all percentage increments. For example, consider the percentage increments and the corresponding incubation times for the seeds of the present study, and for the maintained temperature 24.5° (fig. 5). The six increment-time products are $6.5 \times 18 = 117.0$, $15.1 \times 20 = 302$, $15.8 \times 22 = 347.6$, $14.2 \times 24 = 340.8$, $11.8 \times 26 = 306.8$, and $7.2 \times 28 = 201.6$. The sum of these products (1615.8) is divided by 70.6 (the sum of all six increments, or the final germination percentage) and the resulting quotient is 22.9 hours, which is the required mean incubation time for all tests at 24.5° carried out in this study. These individuals may therefore be said to have required, on the average, an incubation period of 22.9 hours for germination at 24.5° and under the background conditions of the experimental technique employed in these tests.

This mean has different values according to the five different maintained temperatures employed: for 24.5° it is 22.9 hours; for 28.5°, 19.4 hours; for 33.0°, 17.5 hours; for 36.5°, 18.1 hours; and for 40.0°, 22.8 hours. These values are slightly greater than the corresponding incubation times represented by the peaks of the graphs of figure 5, and when plotted their graph is very similar in form to the graphs of figure 4.

Because this index of germination varies with the maintained temperature used in the experiment from which it is derived, it may furnish an additional criterion for establishing a temperature optimum for the germination of a stock of seed, that temperature being taken as optimal for which the lowest mean incubation time is shown. Thus from the values

just given, it appears again that a temperature optimum may be considered as between 33.0° and 36.5°; the difference between the two index values 17.5 (33.0°) and 18.1 (36.5°) probably being insignificant.

When several stocks of seed are to be compared as to average promptness of germination, they might be tested severally in a manner similar to that of this study and a score number or coefficient of germination capacity for each stock might be the mean incubation time corresponding to the optimal temperature for that stock; *i.e.*, the shortest mean incubation times shown by the several stocks, or the reciprocals of these time values, might be useful indices for the comparison of different stocks. Many other uses for this mean incubation time might be suggested.

Summary

1. Samples of a stock of seed of Black Eyebrow soy bean were incubated on 0.5-per cent. agar plates at five maintained temperatures (24.5°, 28.5°, 33.0°, 36.5°, and 40.0°) and the number of germinated seeds recorded at 2-hour intervals, until about 80 per cent. of all the seeds of a test had germinated. According to several different criteria, the optimal temperature range for germination was found to be 33.0°–36.5°. Within this range (a) the least time was required for the appearance of any given germination percentage, (b) the greatest percentage of seeds germinated during any of the incubation periods tested, and (c) the mean incubation time was shortest.

2. The rate of seedling production, measured as the percentage of the total seed population germinating per 2-hour interval, showed a maximum for the third 2-hour interval in which any seedlings were found. For each of the five temperatures tested the germination percentage attained when the rate of seedling production began to fall off was found to be 35–40. For later incubation intervals the rate of seedling production decreased more slowly than it had increased prior to the attainment of its maximal value.

3. The removal of germinated seeds from test cultures at regular intervals constitutes a sorting process which distributes the seedlings derived from a seed sample into several classes, according to their rates of emergence under specified uniform and favorable sets or complexes of germination conditions. In terms of this concept, the increments of germination percentage from one observation to the next indicate the relative sizes of these physiological classes in the original seed sample as well as in the resulting lot of seedlings.

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Johns Hopkins University
Baltimore, Md.

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VITA

Thomas Isaac Edwards was born in Whiting, Kansas, August 20, 1907. He majored in botany and chemistry in Baker University, acting as student assistant in chemistry during the last three years of his college course, and received the degree A.B. in June, 1928. He then became student assistant in plant physiology in the Johns Hopkins University, and had charge of the apparatus and general laboratory facilities in the Laboratory of Plant Physiology from July, 1928, to June, 1932. He specialized in plant physiology throughout his period of residence in the Johns Hopkins University.